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(54) **Oligonucleotides for detecting bacteria.**

(57) Oligonucleotides (SEQ ID NOs 1-8) selectively hybridizable with a specific gene of Vibro parahaemolyticus, oligonucleotides (SEQ ID NOs 9-13) selectively hybridizable with the LT gene of toxigenic Escherchia coil, oligonucleotides (SEQ ID NOs 14-21) selectively hybrizable with the STh or STp gene of toxigenic Escherchia coil, oligonucleotides (SEQ ID NOs 22-47) selectively hybridizable with the entA, B, C, or D gene of Staphylococcus aureus, or oligonucleotides (SEQ ID NOs 48-53) selectively hybridizable with the entE gene of Staplyloccus aureus are prepared and used as primers for gene amplification to thereby selectively detect only respective microorganisms causing food poisoning.

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102 1, 2, 10, 11, 17

This invention relates to means for detecting Vibrio parahaemolyticus, [redacted]man thermostable enterotoxin (hereinafter, STh)- [redacted]li, and Staphylococcus aureus in clinical examination, in particular testing in case of food poisoning, or in food inspection.

In traditional techniques for detection of bacteria, when feces, food, or wipe, a series of operations, namely enrichment culture, isolation culture and differential culture, are required for final identification of the pathogen or contaminant as Vibrio parahaemolyticus, if present. The time periods required for the respective culture steps are 10-16 hours for enrichment culture, 18-24 hours for isolation culture, and 18-24 hours for differential culture, the total time being as long as 2-4 days. Tests to be included in differential culture include growth test in agar medium supplemented with NaCl, gram staining, oxydase test and so forth. They involve complicated and troublesome procedures and are time-consuming and expensive.

For detecting the pathogenic factor of Vibrio parahaemolyticus, the so-called reverse passive hemagglutination reaction is available which uses a specific immunoglobulin obtained from an antiserum to thermostable (thermostable) direct hemolysin (TDH) produced by Vibrio parahaemolyticus. However, this reaction needs 20-24 hours until a result is obtained.

As mentioned above, the prior art methods invariably need a very complicated procedure and a long period of time for identification as Vibrio parahaemolyticus, and are not suited for use in clinical laboratory testing, among others, which demands speediness.

Recently, the DNA probe or hybridization techniques, which use oligonucleotides, have been attempted. However, these techniques, which comprise hybridization with oligonucleotide label-modified probes on a membrane or some other support, followed by detection, scarcely have a satisfactory detection sensitivity and selectivity.

Moreover, strains of Vibrio parahaemolyticus that have a novel pathogenic factor, namely TDH-related hemolysin (TRH), which is different from those so far reported, has been discovered recently and, further, it has become clear that the gene coding for TRH includes two types, namely trh1 and trh2, which differ in base sequence from each other. However, no method has been established as yet for directly testing for Vibrio parahaemolyticus strains having this new pathogenic factor.

Traditionally, for identifying a pathogen or contaminant as a toxigenic strain of Escherichia coli, enrichment culture, isolation culture, pure culture and confirmation culture are required and are to be followed further by serological testing, enterotoxin production test and other biochemical tests. Each culture step requires 18-24 hours and the total time, inclusive of the time for subsequent tests, amounts to as long as a week or so.

For detecting thermolabile enterotoxin (hereinafter, LT), kits for detecting enterotoxin which utilize the reverse passive latex agglutination reaction are commercially available. However, since, immunologically, cholera enterotoxin (hereinafter, CT) and LT have common antigenicity, it is difficult to detect in distinction from each other.

Moreover, the samples should be pure cultures already roughly estimated with respect to their identification. The steps preceding and including this rough estimation step require complicated procedures and a long period of time. In addition, the time for working with said kits alone amounts to 20-24 hours.

As mentioned above, the prior art methods for detecting toxinogenic strains of Escherichia coli invariably need very complicated procedures and are time-consuming, hence are not suited for use in clinical laboratory testing, among others, which demands speediness.

Recently, the DNA probe or hybridization techniques, which use olgonucleotides, have been attempted. However, these techniques, which comprises hybridization with oligonucleotide label-modified probes on a membrane or some other support, followed by detection, scarcely have a satisfactory detection sensitivity and selectivity.

For detecting and identifying STh- or STp-producing toxigenic strains of Escherichia coli, it has been necessary to perform enrichment culture, isolation culture, pure culture and confirmation culture. Furthermore, a pathogen or contaminant can be identified as a thermostable enterotoxin-producing strain of Escherichia coli only after serologic, biochemical, and enterotoxin production tests.

However, 18-24 hours is required for each culture step, and the total time, inclusive of the time for the subsequent tests, amounts to at least one week.

The suckling mouse technique is the only testing method for the production of thermostable enterotoxin. For this method, mice 2-3 days after birth must be prepared and the procedure is complicated and requires skill. Moreover, three or more mice should be subjected to the test to obtain the mean value. For these and other reasons, said method is unsatisfactory in reproducibility and reliability.

Recently, the DNA probe or hybridization techniques, which use oligonucleotides, have been attempted. However, these techniques, which comprise hybridization with oligonucleotide label-modified probes on a

membrane or some other support, followed by detection, scarcely have a satisfactory detection sensitivity and selectivity.

The materials to be tested in case of food poisoning include patients' vomits, feces, the same foods as taken by patients and/or wipes used in patients' environment. For the detection and identification of Staphylococcus aureus in these materials, it has been necessary to first perform enrichment culture, isolation culture, pure culture and confirmation culture.

However, 18-24 hours is required for each culture step, and the total time, including the time necessary for the subsequent tests, is very long, amounting to about 4 days.

Biochemical tests to be carried out following confirmation culture include, among others tests for aerobic growth, VP reactivity, nitrite reduction, Tween™' 80 hydrolysis, hyaluronidase activity, sugar degradation and so forth. These tests require complicated procedures and are time-consuming and expensive.

In the case of Staphylococcus aureus, testing of isolates for enterotoxin production is regarded as the most reliable method of identifying pathogenic bacteria causative of food poisoning and diarrhea. However, even when commercially available simple reagent kits are used, 18-20 hours is required until results can be obtained. This means lack of speediness. A simple reagent kit for enterotoxin E (see) is not commercially available.

Recently, the DNA probe or hybridization techniques, which use olgonucleotides, have been attempted. However, these techniques, which comprises hybridization with oligonucleotide label-modified probes on a membrane or some other support, followed by detection, scarcely have a satisfactory detection sensitivity and selectivity.

The present invention envisages a simple, speedy and highly sensitive method useful in testing for microorganisms causative of food poisoning, which method comprises detecting a Vibrio parahaemolyticus-derived specific gene by the gene amplification technique using oligonucleotides as primers in the nucleic acid synthesis reaction.

The invention also envisages a simple, speedy and highly sensitive method useful in testing for microorganisms causative of food poisoning, which method comprises detecting a toxigenic Escherichia coli-derived nucleic acid by the gene amplification technique using oligonucleotides as primers in the nucleic acid synthesis reaction.

The present invention further envisages a simple, speedy and highly sensitive method useful in testing for causative microorganisms in case of food poisoning or diarrhea, which method comprises detecting the toxigenic Escherichia coli-derived STh or STp gene by the gene amplification technique using oligonucleotides as primers in the nucleic acid synthesis reaction.

The present invention also envisages a simple, speedy and highly sensitive method useful in testing for causative microorganisms in case of food poisoning or diarrhea, which method comprises detecting the Staphylococcus aureus-derived entA, B, C, D and E genes (enterotoxin A, B, C, D and E gene) by the gene amplification technique using oligonucleotides as primers in the nucleic acid synthesis reaction.

In accordance with a first aspect of the invention, oligonucleotides capable of selectively hybridizing with a specific gene of Vibrio parahaemolyticus are provided and may be used as primers in gene amplification to selectively detect Vibrio parahaemolyticus alone, which causes food poisoning symptoms.

In accordance with a second aspect of the invention, oligonucleotides capable of selectively hybridizing with a toxigenic Escherichia coli-derived nucleic acid are provided and may be used as primers in gene amplification to selectively detect toxigenic Escherichia coli alone, which causes food poisoning symptoms.

In accordance with a third aspect of the invention, oligonucleotides capable of selectively hybridizing with the STh and/or STp gene of toxigenic Escherichia coli are provided and may be used as primers in gene amplification to selectively detect STh- or STp-producing toxigenic Escherichia coli alone from among pathogenic Escherichia coli strains causing food poisoning symptoms.

In accordance with a fourth aspect of the invention, oligonucleotides capable of selectively hybridizing the entA, B, C, D and E genes of Staphyloccocus aureus are provided and may be used as primers in gene amplification to thereby selectively detect entA, B, C, D and E-producing Staphylococcus aureus alone from among Staphylococcus aureus strains causing food poisoning symptoms.

Oligonucleotides which may be used as primers in accordance with the first aspect of the invention, when intended for detecting the type 1 and type 2 thermostable hemolysin-related hemolysin genes (trh1 and trh2 genes), may be oligonucleotides having the sequences

(5') d-GGCTCAAAATGGTTAAGCG (3') (a)
(5') d-CATTTCCGCTCTCATATGC (3') (b)

or the corresponding complementary sequences.

When intended for detecting the thermostable hemolysin gene (tdh gene), they may be oligonucleotides having the sequences

(5') d-CCATCTGTCCCTTTTCCTGC (3') (c)
(5') d-CCAAATACATTTTACTTGG (3') (d)
(5') d-GGTACTAAATGGCTGACATC (3') (e)
(5') d-CCACTACCACTCTCATATGC (3') (f)

or the corresponding complementary sequences.

Further, when intended for detecting the thermostable hemolysin-related hemolysin gene type 1 (trh1 gene) of Vibrio parahaemolyticus, they may be oligonucleotides having the sequences or the corresponding complementary sequences.

The gene amplification can be effected by the polymerase chain reaction method developed by Saiki et al. [hereinafter, PCR method for short; Science, 230, 1350 (1985)]. This method comprises preparing two oligonucleotides, one recognizing and hybridizing with the + chain and the other recognizing and hybridizing with the - chain at the ends of a specific nucleotide sequence region to be detected (in the present case, the trh gene or tdh gene, or trh1 gene of Vibrio parahaemolyticus). In this method, these function as primers for the template-dependent nucleotide polymerization reaction against the sample nucleic acid, in the single stranded form as a result of heat denaturation. The resulting double-stranded nucleic acid is separated into single strands allowing the hybridizing of primers followed by primer extension to be repeated. By repeating this serial procedure, the number of copies of the region between the two primers is increased so that said region can be detected.

The sample to be tested may be a laboratory test sample such as feces, urine, blood or tissue homogenate, or a food sample.

For testing such samples using PCR, pretreatment is necessary by which the nucleic acid components are released from microbial cells occurring in the sample.

Since, however, the PCR can proceed if only a small number of molecules of the nucleic acid hybridizable with the primers are present, a short period of treatment of the laboratory sample with a lytic enzyme, a surfactant, an alkali or the like can give a sample solution containing the nucleic acid in a sufficient amount to enable the PCR to proceed.

The oligonucleotides which may be used as primers in the practice of the invention are nucleotide fragments which may have a length of 10 bases or more, desirably 15 bases or more, from the viewpoints of selectivity, detection sensitivity and reproducibility. They may be chemically synthesized ones or natural ones. The primers need not be particularly labeled for detection purposes. The primers may comprise part of any one of the nucleotide sequences given in this application, provided they maintain the desired target specificity.

The primer specified amplification region of the nucleotide sequence of a particular gene of Vibrio parahaemolyticus may comprise 50 bases to 2,000 bases, desirably 100 bases to 1,000 bases.

In carrying out the template-dependent nucleotide polymerization reaction, a thermostable DNA polymerase may be used. This enzyme may be of any origin provided that it can retain its activity at temperatures of 90-95 °C. The heat denaturation is usually carried out at 90-95 °C, the annealing for primer hybridization at 37-65 °C, and the polymerization reaction at 50-75 °C, and these constitute one cycle of PCR. Amplification is effected by repeating PCR cycles, perhaps 20-42 cycles.

For detection, the reaction mixture after completion of the PCR may be subjected, as such, to agarose gel electrophoresis, whereby the presence or absence of the amplified nucleotide fragment and, if present, the length thereof can be confirmed. Based on the results, judgment can be made as to whether the nucleotide having the sequence to be recognized by the primers is present in the sample or not. This judgment directly serves as a judgment as to whether Vibrio parahaemolyticus carrying the trh gene or the like is present or not. Other electrophoretic techniques and chromatographic techniques are also effective in detecting the amplified nucleotide fragment.

The oligonucleotides to be used in accordance with the second aspect of the invention are oligonucleotides the target of which is a nucleotide sequence coding for the heat-labile toxin (LT) that is produced by toxigenic Escherichia coli occurring in the sample. They are complementary to said nucleotide sequence and may have the following sequences

(5') d-CCCAGATGAAATAAAACGT-(3') (a)
(5') d-CCTGAGATATATTGTGCTC-(3') (b)
(5') d-ACAAACCGGCTTTGTCAGATAT-(3') (c)
(5') d-GTTATATATGTCAACCTCTGAC-(3') (d)
(5') d-ACCGGTATTACAGAAATCTGA-(3') (e)

or the corresponding complementary sequences.

The gene amplification may be carried out based on the PCR method mentioned above.

The samples to be used and the method of pretreatment thereof may be the same as mentioned above.

The oligonucleotides to be used as primers in accordance with the second aspect of the invention each may have the same length as mentioned above with respect to the first aspect of the invention. They may be chemically synthesized ones or natural ones. The primers need not be particularly labeled for detection purposes.

The primer-specified amplification region in the nucleotide sequence of a specific gene of toxigenic Escherichia coli may be 50 to 2,000 bases, desirably 100 to 1,000 bases, in length, as mentioned above.

The template-dependent nucleotide polymerization reaction may be carried out in the same manner as in the first aspect of the invention.

The detection can be made in the same manner as in the first aspect of the invention to thereby judge as to whether LT-producing Escherichia coli is present or not. Other electrophoretic techniques, chromatographic techniques and DNA probing techniques are also efficient in detecting the amplified nucleotide fragment.

The oligonucleotides to be used in accordance with the third aspect of the invention are oligonucleotides supplementary to a nucleotide sequence coding for the STh gene and STp gene, when said nucleotide sequence is the target. Said synthetic nucleotides may have the sequences

(5') d-TGTAATTTTCTCTTTTGAAGACTC-(3') (a: SEQ ID NO: 1)
(5') d-ATTACAACACAGTTCACAGCAG-(3') (b: SEQ ID NO: 2)

or the corresponding complementary sequences.

When a nucleotide sequence coding for the STh gene is the target, the above-mentioned oligonucleotides may be oligonucleotides complementary to said nucleotide sequence and may have the sequences

(5') d-CCTCAGGATGCTAAACCAG-(3') (c: SEQ ID NO: 3)
(5') d-AGGATGCTAAACCAGTAGAG-(3') (d: SEQ ID NO: 4)
(5') d-AATTCACAGCAGTAATTGCTAC-(3') (e: SEQ ID NO: 5)

or the corresponding complementary sequences.

rimers pecific each gene.

Furthermore, when a nucleotide sequence coding for the STp gene is the target, they may be oligonucleotides complementary to said nucleotide sequence and may have the sequences

(5') d-TCTTTCCCCTCTTTTAGTCAG-(3') (f: SEQ ID NO: 6)
(5') d-GTCAACTGAATCACTTGACTC-(3') (g: SEQ ID NO: 7)
(5') d-TCACAGCAGTAAAATGTGTTG-(3') (h: SEQ ID NO: 8)

or the corresponding complementary sequences.

The gene amplification may be effected based on the PCR method mentioned above.

The samples to be tested and the method of pretreatment thereof may be the same as those mentioned above with respect to the first aspect of the invention.

The oligonucleotides to be used as primers in accordance with the third aspect of the invention may have the same length as mentioned above with respect to the first aspect of the invention. Thus, they may be nucleotide fragments having a length of 10 or more bases, desirably 15 or more bases, and may be chemically synthesized ones or natural ones.

The primers need not be labeled for particular detection purposes. The primer-specified amplification range in the nucleotide sequence for the STh or STp gene of toxigenic Escherichia coli may be the same in length as mentioned above and thus may cover 50 bases to 2,000 bases, desirably 100 bases to 1,000 bases.

The template-dependent nucleotide polymerization reaction may be carried out in the same manner as mentioned above with respect to the first aspect of the invention.

The detection may be conducted in the same manner as mentioned above with respect to the first aspect of the invention, whereby judgement can be made as to whether pathogenic Escherichia coli carrying the STh or STp gene is present or not. Other electrophoretic techniques and chromatographic techniques are also effective in detecting the amplified nucleotide fragment.

It is also possible to selectively detect the target nucleotide sequence on a membrane or some other support by allowing an oligonucleotide having one of the above sequences (a) to (h) to function as a probe. In this case, said oligonucleotide is preferably modified with a label.

The oligonucleotides to be used as primers in accordance with the fourth aspect of the invention, when a nucleotide sequence coding for the entA gene is the target, may be oligonucleotides complementary to said nucleotide sequence and with the sequences

(5') d-GTCTGAATTGCAGGGAACAG-(3') (a)
(5') d-CTTTTTTACAGATCATTCGTG-(3') (b)

(5') d-TAGATTTTGATTCAAAGGATATTG-(3') (c)
(5') d-CTTATTCGTTTTAACCGTTTCC-(3') (d)
(5') d-AACACGATTAATCCCCTCTG-(3') (e)
(5') d-TCGTAATTAACCGAAGGTTCTG-3') (f)
or the corresponding complementary sequences.

When a nucleotide sequence coding for the entB gene is the target, they may be oligonucleotides complementary to said nucleotide sequence and may have the sequences
(5') d-AAATCTATAGATCAATTTCTATAC-(3') (g)
(5') d-AATTATGATAATGTTCGAGTCG-(3') (h)
(5') d-TTCGCATCAAACTGACAAACG-(3') (i)
(5') d-CATCTTCAAATACCCGAACAG-(3') (j)
(5') d-CCAAATAGTGACGAGTTAGG-(3') (k)
(5') d-TCATACCAAAAGCTATTCTCAT-3') (l)
or the corresponding complementary sequences.

Further, when a nucleotide sequence coding for the entC gene is the target, they may be oligonucleotides complementary to said nucleotide sequence and may have the sequences
(5') d-TCTGTAGATAAATTTTTGGCA-(3') (m)
(5') d-AAAATTATGACAAAGTGAAAACAG-(3') (n)
(5') d-ATGGATCAAATTACTATGTAAAC-(3') (o)
(5') d-GTAGGTAAAGTTACAGGTGG-(3') (p)
(5') d-TATAAGTACATTTTGTAAGTTCC-(3') (q)
(5') d-CATACCAAAAAGTATTGCCGTT-(3') (r)
or the corresponding complementary sequences.

When a nucleotide sequence coding for the entD gene is the target, they may be oligonucleotides complementary to said nucleotide sequence and may have the sequences
(5') d-AAAATCTGAATTAAGTAGTACCG-(3') (s)
(5') d-ATAGGAGAAAATAAAAGTACAGG-(3') (t)
(5') d-CTTCAATTCAAAAGAAATGGC-(3') (u)
(5') d-TTGTACATATGGAGGTGTCAC-(3') (v)
(5') d-TTTTAGATTTGAAATGTTGAGCC-(3') (w)
(5') d-TGACACCTCCATATGTACAAG-(3') (x)
(5') d-ATTATACAATTTTAAATCCTTTTGC-(3') (y)
(5') d-CTGTATTTTTCCTCCGAGAGT-(3') (z)
or the corresponding complementary sequences.

When a nucleotide sequence coding for the entE gene is the target, they may be oligonucleotides complementary to said nucleotide sequence and may have the sequences
(5')d-AAAAGTCTGAATTACAAAGAAATG-(3') (a:SEQ ID NO;48),
(5')d-GGTTTTTTCACAGGTCATCCA-(3') (b:SEQ ID NO;49),
(5')d-GAACAGTTACTTCTTTTTTGCTT-(3') (c:SEQ ID NO;50),
(5')d-CTGTCTGAGTTATATAAACCAA-(3') (d:SEQ ID NO;51),
(5')d-GCACCTTACCGCCAAAGCTG-(3') (e:SEQ ID NO;52),
(5')d-AAACAAATCATAACTTACCGTG-(3') (f:SEQ ID NO;53),
or the corresponding complementary sequences.

The gene amplification may be carried out based on the PCR method mentioned above.

The samples to be tested and the method of pretreatment thereof may be the same as those mentioned above with respect to the first aspect of the invention.

The oligonucleotides to be used as primers in accordance with the fourth aspect of the invention may have the same length as mentioned above with respect to the first aspect of the invention. They may be chemically synthesized ones or natural ones.

The primers need not be particularly labeled for detection purposes. The primer-specified amplification range in the nucleotide sequence for the entA, B, C, D, or E gene of Staphylococcus aureus may cover 50 to 2,000 bases, desirably 100 to 1,000 bases, as in the cases mentioned above.

The template-dependent nucleotide polymerization reaction may be carried out in the same manner as mentioned above with respect to the first aspect of the invention.

The detection may be performed in the same manner as in the first aspect of the invention, whereby judgment can be made as to whether Staphylococcus aureus having the entA, B, C, D, or E gene is pressent or not. Other electrophoretic techniques and chromatographic techniques are also effective in detecting the amplified oligonucleotide fragment.

It is also possible to selectively detect the target oligonucleotide on a membrane or some other support by allowing an oligonucleotide having one of the sequences (a) to (z) (SEQ ID NOs 22-47) and (a) to (f) (SEQ ID NOs 48-53) given above as a probe. In this case, said oligonucleotide is preferably modified with a label.

As mentioned above, the present invention makes it possible to detect Vibrio parahaemolyticus, toxinogenic Escherichia coli, or Staphylococcus aureus in a simple and speedy manner and with high sensitivity by detecting a Vibrio parahaemolyticus-derived specific gene, the toxinogenic Escherichia coli-derived LT gene, a toxinogenic Escherichia coli-derived specific gene or a specific gene of Staphylococcus aureus by the gene amplification technique using certain oligonucleotides as primers in the nucleic acid synthesis reaction. The present invention will now be illustrated by exemplification.

EXAMPLE 1

Detection of the trh gene of Vibrio parahaemolyticus
(Experiment 1)

Sample preparation

A total of 326 strains of Vibrio parahaemolyticus and other Vibrio species as specified in Tables 1 to 15 under the columns "Strain" and "Strain No." were used. Each was inoculated into an appropriate enrichment medium and incubated overnight at 37°C under aerobic conditions. Cells were recovered from the medium (1.5 ml) by centrifugation. They were washed once with 10 mM Tris-hydrochloride buffer (pH 7.5) and then subjected to lysis by suspending them in 0.5 ml of a 1 mg/ml lysozyme solution in the same buffer at 37°C for 10 minutes. A phenol-chloroform mixture (mixing ratio 1:1) saturated with the above-mentioned buffer was added to the lysate solution, followed by thorough stirring. After centrifugation, the upper layer was recovered and subjected to ethanol treatment for precipitating nucleic acid components. The precipitate was dissolved in 1 ml of the above-mentioned buffer and the solution was used as a sample.

Primer synthesis

Based on the base sequence of the trh gene of Vibrio parahaemolyticus [Nishibuchi, M. et al.: Infect. Immun., 57, 2691-2697 (1989) and Kishishita et al.: Japan. J. Bacteriol., 45, 340 (1990)], the sequences specifically shown in Claim 1 were selected and oligonucleotides identical in sequence therewith were chemically synthesized. The chemical synthesis was carried out by the β-cyanoethylphosphamidite method using a Cyclone Plus DNA synthesizer (Milligen/BioResearch). The oligonucleotides synthesized were purified by high-performance liquid chromatography using a C18 reversed phase column.

PCR

A reaction mixture (30 μl) was prepared by adding, to 3 μl of the sample solution mentioned above, 17.55 μl of sterile distilled water, 3 μl of 10 x reaction buffer, 4,8 μl of dNTP solution, 0.75 μl of primer (a), 0.75 μl of primer (b) and 0.15 μl of thermostable DNA polymerase. Mineral oil (50 μl; Sigma) was layered on said reaction mixture placed in a container. The solutions used in preparing the reaction mixture were as shown below.

10 x Reaction buffer: 500 mM KCl, 100 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$, 0.1% (w/v) gelatin.

dNTP solution: containing dATP, dCTP, dGTP and dTTP each at a final concentration of 1.25 mM.

Primer (a) and primer (b): Each was an aqueous solution of each product chemically synthesized and purified as mentioned above (concentration 5 ODU/ml).

Thermostable DNA polymerase: Taq DNA polymerase (5 units/ml; Perkin Elmer Cetus)

The reaction conditions were as follows.

Heat denaturation: 94°C, 1 minute.

Annealing: 55°C, 1 minute.

Polymerization reaction: 72°C, 1 minute.

The time require for each cycle covering the process from heat denaturation, through annealing, to polymerization reaction was 5.7 minutes. This cycle was repeated 35 times (total time required about 3 hours). For carrying out these operations, the above reaction conditions were programmed into a DNA Thermal Cycler apparatus (Perkin Elmer Cetus).

Detection

For detecting the amplified nucleotide fragment in the reaction mixture, agarose gel electrophoresis was performed in the following manner.

The agarose gel used had a gel concentration of 2% (w/v) and contained ethidium bromide (0.5 μl/ml). The electrophoresis was conducted at a constant voltage of 100 V for 30 minutes. The procedure and other conditions were as described in Maniatis et al.: Molecular Cloning (1982). In addition to the reaction mixture, molecular weight markers were simultaneously electrophoresed and, based on the comparison of relative mobilities, the length of the nucleotide fragment was estimated.

Results

As mentioned hereinabove, the base sequence of the trh gene of Vibrio parahaemolyticus has already been determined and two types differing in base sequence, namely trh1 and trh2 have been reported. Therefore, the size of the nucleotide amplified by the PCR using the oligonucleotides, namely primers, of the present invention can be estimated. Thus, with primers (a) and (b), the nucleotide amplified is expected to be 251 bases long. When the size of the nucleotide amplified was in agreement with such estimated value, then it was judged that each primer contributed to correct amplification of the target region in the trh gene. The results obtained with 264 strains of Vibrio parahaemolyticus and 98 strains of other Vibrio species are shown in Table 1. As can be seen from Table 1, the primers could correctly detect both the trh1 and trh2 genes of Vibrio parahaemolyticus.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for Vibrio parahaemolyticus strains having the trh1 or trh2 gene, diarrhea-causing microorganisms other than Vibrio haemolyticus but generally included as targets in clinical laboratory testing were further tested for comparison.

The method was the same as shown in Experiment 1 except that the strains listed in Tables 16 and 17 under Nos. 12, 13, 19 and 20 were cultured overnight at 37°C under anaerobic conditions for preparing samples submissible to the PCR method. A total of 39 strains listed in Table 16 and 17 were cultured for preparing samples. The human placenta-derived DNA was diluted to a concentration of 1 μg/ml and subjected to PCR in the same manner. The results thus obtained are shown in Tables 16 and 17. Some microorganisms gave amplified nucleotide fragments, which were presumably byproducts of the PCR All of said fragments differed in size from the nucleotide fragment anticipated from the sequence of the trh1 or trh2 gene. If these diarrhea-causing microorganisms had the same trh gene as that of Vibrio parahaemolyticus, the same 251-base-long nucleotide fragment as found in Experiment 1 would have been detected. It is therefore clear that the amplified nucleotides detected for some diarrhea-causing microorganisms were not the products resulting from recognition of the trh gene of Vibrio parahaemolyticus but that trh gene-carrying strains of Vibrio parahaemolyticus and other Vibrio species can be readily detected in distinction from other diarrhea-causing microorganisms. The agarose gel electrophoresis employed in the experiment described herein, when conducted under the conditions mentioned above, can distinguish nucleotides of 100 base pairs or less in size from each other when they differ by 5 to 10 base pairs, and uncleotides of 100 to 500 base pairs in size from each other when they differ by 10 to 20 base pairs. When the precision of nucleotide size determination is improved by using an acrylamide gel, for instance, the reliability in the selective detection will presumably be increased further.

EXAMPLE 2

Detection of the tdh gene of vibrio parahaemolyticus

Experiment 1

Sample preparation

Samples were prepared in the same manner as in Example 1.

Primer synthesis

Based on the base sequence of the rdh gene of Vibrio parahaemolyticus [Nishibuchi, M. and kaper, J. B.: Mol. Microbiol., 4, 87-99 (1990)], the sequences shown in Claim 2 were selected, and oligonucleotides having the same sequences as those were chemically synthesized. The chemical synthesis and purification of the oligonucleotides synthesized were carried out in the same manner as in Example 1.

PCR

The same procedure as used in Example 1 was followed except that the following primer combinations were used.

Primer (1)	Primer (2)
(c)	(d)
(e)	(d)
(e)	(f)

Detection

The same procedure as used in Example 1 was followed.

Results

As mentioned above, the base sequence of the tdh gene of Vibrio parahaemolyticus has already been determined. Therefore, the size of the uncleotide amplified by the PCR using the oligonucleotides, or primers, of the present invention can be estimated. Thus, when primers (c) and (d), (e) and (d), or (e) and (f) are used, the oligonucleotide amplified is expected to be 373, 199, or 251 bases long, respectively. When the size of the amplified uncleotide was in agreement with such estimated value, it was judged that each primer contributed to correct amplification of the target region in the tdh gene. The results thus obtained with 264 strains of Vibrio parahaemolyticus and 98 strains of other Vibrio species are shown in Tables 1-15. As can be seen from Tables 1-15, the three primer combinations each could correctly detect tdh gene-containing strains alone among the strains of Vibrio parahaemolyticus and Vibrio species tested.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for tdh gene-carrying strains of Vibrio parahaemolyticus and Vibrio species, diarrhea-causing microorganisms other than Vibrio parahaemolyticus but generally included as targets in clinical laboratory testing were tested for comparison in the same manner as in Example 1.

The results obtained are shown in Tables 16 and 17. In some microorganisms, amplified nucleotide fragments, which were presumably byproducts of the PCR, were detected.

However, they all differed in size from the nucleotide fragment anticipated from the sequence of the tdh gene. If these diarrhea-causing microorganisms had the same tdh gene as that of Vibrio parahaemolyticus, a nucleotide fragment identical in size as that detected in Experiment 1, namely 373, 199 or 251 bases long, would have been detected. It is therefore evident that the amplified nucleotides observed for said certain diarrhea-causing microorganisms were not the products resulting from recognition of the tdh gene of Vibrio parahaemolyticus but that those stains of Vibrio parahaemolyticus and Vibrio species that have the tdh gene can be readily detected in distinction from other diarrhea-causing microorganisms.

EXAMPLE 3

Detection of the trh1 gene of Vibrio parahaemolyticus

Experiment 1

Sample preparation, primer synthesis, PCR and detection were carried out in the same manner as in Example 1 except that the primers (g) and (h) defined in Claim 3 were used.

When the primers (g) an (h) of the invention are used, it is expected that a nucleotide 211 bases long be amplified. When the size of the nucleotide amplified was in agreement with said estimated value, it was judged that each primer contributed to correct amplification of the target region in the trh1 gene. The results obtained with 264 strains of Vibrio parahaemolyticus and 98 strains of Vibrio species are shown in Tables 1-15. As can be seen in Tables 1-15, the primer combination could correctly detect only those strains having the trh1 gene among the strains of Vibrio parahaemolyticus and Vibrio species.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for the trh1 gene-containing strains of Vibrio parahaemolyticus and Vibrio species, diarrhea-causing microorganisms other than Vibrio parahaemolyticus but generally involved as targets in clinical laboratory testing were tested for comparison by the same method as used in Example 1.

The results obtained are shown in Tables 16 and 17. With some microorganisms, amplified nucleotide fragments, which were presumably byproducts of the PCR, were detected.

However, none of them was identical in size with the nucleotide fragment anticipated from the sequence of the trh1 gene. If these diarrhea-causing microorganisms had the same trh1 gene as that of Vibrio parahaemolyticus, the same, 211-base-long nucleotide fragment as detected in Experiment 1 would have been detected. Therefore, it is evident that the amplified nucleotides found in said certain diarrhea-causing microorganisms were by no means the products of recognition of the trh1 gene of Vibrio parahaemolyticus but that trh1 gene-containing strains of Vibrio parahaemolyticus can be readily detected in distinction from other diarrhea-causing microorganisms.

Tables 1-17

Notes to the above Tables:

(1) in column "trh': strain having the type 1 trh gene.
(2) in column "trh': strain having the type 2 trh gene.
(-) in column "trh': strain having no trh gene.
(+) in column "tdh': strain having the tdh gene.
(-) in column "tdh': strain having no tdh gene.
(O) in column "Primer combination": The amplified nucleotide had the same size as the expected value.
(•) in column "Primer combination": The amplified nucleotide, though detected, had a size different from the expected value.
(-) in column "Primer combination": No amplified nucleotide was detected.

The strains shown in Tables 1-15 were obtained from; Department of Microbiology, Faculty of Medicine, Kyoto University.

The strains shown in Tables 16 an 17 were obtained from: ATCC (American Type Culture collection), JCM (Japanese Collection of Microorganisms, RIKEN Institute of Physical and Chemical Research), and IFO (Institute for Fermentation, Osaka).

EXAMPLE 4

Detection of the LT gene of toxigenic Escherichia coli

Experiment 1

Sample preparation

For screening toxigenic strains of Escherichia coli, 492 clinical isolates from patients with diarrhea, shown in Tables 18-30, were used. These strains were gifts from the Department of Microbiology (chief: Prof. Y. Takeda), Faculty of Medicine, Kyoto University. Each strain was inoculated into an appropriate medium and cultured overnight at 37 °C under aerobic conditions. Cells were recovered from the medium (1.5 ml) by centrifugation, washed once with 10 mM Tris-hydrochloride (pH 7.5) and then subjected to lysis by suspending them in 0.5 ml of a 1 mg/ml lysozyme solution in the same buffer at 37 °C for 10 minutes. A phenol-chloroform mixture (mixing ratio 1:1) saturated with the above-mentioned buffer was added to the lysate solution, followed by thorough stirring. After centrifugation, the upper layer was recovered and subjected to ethanol treatment for precipitating nucleic acid components. The precipitate was dissolved in 1 ml of the above-mentioned buffer and the solution was used as a sample.

Primer synthesis

Based on the base sequence of the LT gene of heat-labile enterotoxin-producing Escherichia coli - [Yamamoto, T., T. Tamura and T. Yokota (1984): Primary structure of heat-labile enterotoxin produced by Escherichia coli pathogenic for humans. J. Biol. Chem., 259: 5037-5044 and Yamamoto, T., T. Gojobori and T. Yokota (1987): Evolutionary origin of pathogenic determinants in enterotoxigenic Escherichia coli and Vibrio cholerae OI. J. Bacteriol., 169: 1352-1357], the sequences (a), (b), (c), (d) and (e) shown in Claim 5 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis was carried out by the β-cyanoethylphosphamidite method using a Cylcone Plus DNA synthesizer (Milligen/BioResearch). The oligonucleotides synthesized were purified by high-performance liquid chromatography using a C18 reversed phase column.

PCR

A reaction mixture (30 μl) was prepared by adding, to 3 μl of the sample solution mentioned above, 16.05 μl of sterile water, 3 μl of 10 x reaction buffer, 4.8 μm of dNTP solution, 1.5 μl of primer (1), 1.5 μl of primer (2), and 0.15 μl of thermostable DNA polymerase. Mineral oil (50 μl; Sigma) was layered on said reaction mixture placed in a container. The solutions used in preparing the reaction mixture and the combination of primers (1) and (2) were as shown below.

10 x Reaction buffer: 500 mM KCl, 100 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$, 0.1% (w/v) gelatin.

dNTP solution: containing dATP, dCTP, dGTP and dTTP each at a concentration of 1.25 mM.

Primers (1) and (2): Each was an aqueous solution of each product chemically synthesized and purified as mentioned above (concentration 5 ODU/ml).

Primer combination: The following three combinations of the chemically synthesized and purified products mentioned above were used.

Primer (1)	Primer (2)
(a)	(b)
(c)	(d)
(e)	(d)

Thermostable DNA polymerase: Taq DNA polymerase (5 units/ml; Perkin Elmer Cetus).

The reaction conditions were the same as in Example 1.

Detection

For detecting the amplified nucleotide fragment in the reaction mixture, agarose gel electrophoresis was performed under the same conditions as used in Example 1 except that the agarose gel had a gel

concentration of 3% (w/v).

Results

As mentioned above, the base sequence of the LT gene has already been determined. Therefore, the size of the nucleotide amplified by the PCR using the oligonucleotides, or primers, of the invention can be estimated. Thus, the primer combination (a) + (b), (c) + (d) and (e) + (d) are expected to give amplified nucleotides of 589, 550 and 264 bases in size, respectively. When the size of the amplified nucleotide was in agreement with the estimated value, it was judged that each primer contributed to correct amplification of the target region in the LT gene. The results of testing of 492 clinical isolates of Escherichia coli for nucleotide amplification by the above method are shown in Tables 18-30. In Tables 18-30, the symbol + in the column "primer" means that the size of the amplified nucleotide agreed with the estimated value while the symbol - means that no amplified nucleotide was detected at all.

As shown in the tables, the PCR conducted with each primer combination resulted in nucleotide amplification only in the strains having the LT gene (those marked with +, 2+ or w in the LT gene column in Tables 18-30). Furthermore, each amplified nucleotide had the same nucleotide size as estimated. It is therefore evident that the oligonucleotides, namely primers, of the invention could contribute to correct amplification of the target region in the LT gene of toxigenic Escherichia coli.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for LT-producing strains of Escherichia coli, microorganisms other than Escherichia coli but generally included as targets in clinical laboratory testing were also tested for comparison.

The method used was the same as shown in Example 1 except that, for Clostridium perfringens, Campylobacter jejuni, Campylobacter coli, Bacteroides flagilis, Bacteroides vulgatus and Lactobacillus acidophilus, samples applicable to the PCR method were prepared by overnight culture at 37 °C under anaerobic conditions. As shown in Tables 31 and 32, 50 microbial strains were cultured for preparing samples. Further, a 1 μg/ml solution of human placenta DNA was prepared and also subjected to PCR.

The results obtained are shown in Tables 31 and 32. None of the three primer combinations used caused amplification of DNA of various bacteria other than pathogenic Escherichia coli. It is therefore declarable that the oligonucleotides, namely primers, of the invention react selectively with heat-labile enterotoxin-producing Escherichia coli alone.

On the other hand, it is clear that, in accordance with the invention, no cross reaction occurs with CT-producing Vibrio cholerae (in Table 32, V. cholerae 01 ctx+). Immunologically, CT and LT have common antigenicity, as mentioned above, and any immunological means cannot distinguish them from each other. On the contrary, the method according to the invention can distinctly detect LT-producing Escherichia coli alone, hence can be considered to show improved reliability as compared with the prior art methods.

The agarose gel electrophoresis described herein in the examples, when carried out under the conditions mentioned above, can distinguish nucleotides of 100 or less base pairs in size from each other when they differ in size by 5 to 10 base pairs, and nucleotides of 100 to 500 base pairs in size from each other when they differ in size by 10 to 20 base pairs. The precision of nucleotide size measurement can be improved by using acrylamide, for instance, as the gel and, by doing so, the reliability in the selective detection of the LT gene can probably be further improved.

Table 18 - Table 32

Notes to the above tables:

(+) in column "LT gene" :	The strain has the LT gene.
(2+) in column "LT gene":	The strain has the LT gene.
(w) in column "LT gene" :	The strain has the LT gene.
(-) in column "LT gene" :	The strain has no LT gene.
(+) in column "Primer" :	The size of the amplified nucleotide is in agreement with the estimated value.
(w) in column "Primer" :	The size of the amplified nucleotide is in agreement with the estimated value but the extent of amplification is somewhat weak.
(-) in column "Primer" :	No nucleotide amplification was noted at all.

The strains shown in Tables 18-30 were obtained from:
Department of Microbiology, Faculty of Medicine, Kyoto University.
The strains Nos. 1-39 shown in Tables 31 and 32 were obtained from:
ATCC (American Type Culture Collection),
JCM (Japanese Collection of Microorganisms, RIKEN Institute of Physical and Chemical Research), and
IFO (Institute for Fermentation, Osaka).
The strains Nos. 4-50 shown in Table 32 were obtained from:
Department of Microbiology, Faculty of Medicine, Kyoto University.
The strains No. 51 shown in Table 32 was obtained from:
Takara Shuzo Co., Ltd.

EXAMPLE 5

Detection of the RTh or RTp gene of toxigenic Escherichia coli

Experiment 1

Sample preparation

As shown in Tables 33-54, a total of 492 strains of pathogenic Escherichia coli as isolated from patients with diarrhea were used. Each strain was inoculated into an appropriate enrichment medium and cultured overnight at 37°C under aerobic conditions. Each culture broth was diluted with 10 mM Tris-hydrochloride buffer (pH 7.5) (hereinafter, TE buffer), heat-treated at 95°C for 10 minutes and then centrifuged. The supernatant was used as a sample.

Primer synthesis

Based on the base sequence of the STh or STp gene of toxigenic Escherichia coli [Moseley, S. L., et al., Infect. Immun., 39, 1167-1174 (1983)], the sequences (a) and (b) defined in Claim 7 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis was carried out by the β-cyanoethylphosphamidite method using a Cyclone Plus DNA synthesizer (Milligen/BioResearch). The oligonucleotides synthesized were purified by high-performance liquid chromatography using a C18 reversed phase column.

PCR

The procedure of Example 4 was followed.
The combination of primers (1) and (2) was as follows.
Primers (1) and (2): Each an aqueous solution of the chemically synthesized and purified product mentioned above (concentration 5 OD/ml).
Primer combination: primer (1): sequence (a),
primer (2): sequence (b).
Thermostable DNA polymerase: Taq DNA polymerase (5 units/ml; Perkin Elmer Cetus).
The reaction conditions were the same as used in Example 1.

Detection

The procedure of Example 4 was followed.

Results

As mentioned above, the base sequence of the STh or STp gene of toxigenic Escherichia coli has already been determined and, therefore, the size of the nucleotide to be amplified by he PCR using the oligonucleotides, namely primers, of the invention can readily be estimated.

Thus, when the combination of primers (a) and (b) is used, a nucleotide of 120 bases in size is expected to be amplified. When the size of the amplified nucleotide was in agreement with said estimated value, it was judged that each primer could contribute to correct amplification of the target region in the STh or STp gene. This judgment was indicated by "+" in Tables 33-54. When no nucleotide amplification was

noted, this result was indicated by "-".

The results obtained with 492 strains of pathogenic Escherichia coli are shown in Tables 33-54. As can be seen in Tables 33-54, the primers used could correctly detect only those strains shown to have the STh or STp gene by the colony hybridization technique among the pathogenic Escherichia coli strains tested.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for pathogenic Escherichia coli strains having the STh or STp gene, diarrhea-causing microorganisms other than pathogenic Escherichia coli but generally included as targets in clinical laboratory testing were also tested for comparison.

The procedure of Example 1 was followed except for the method of sample preparation, which is mentioned below.

Sample preparation

Each of the strains shown in Tables 55-57 was inoculated into an appropriate enrichment medium and cultured overnight at 37°C under aerobic or anaerobic conditions (strains cultured under anaerobic conditions were Clostridium perfringens, Campylobacter jejuni, Bacteroides flagilis, Bacteroides vulgatus and Lactobacillus acidophilus).

Cells were recovered from each culture broth (0.5 ml) by centrifugation and washed once with TE buffer. To the cells were added an N-acetylmuramidase solution in 50 mM phosphate buffer (pH 7.5) and an achromopeptidase solution to final concentrations of 50 µg/ml and 1 mg/ml, respectively, and the mixture was treated at 37°C for 10 minutes for causing lysis. A phenol-chloroform mixture (mixing ratio 1:1) saturated with TE buffer was added to the lysate, followed by thorough stirring.

After centrifugation, the upper layer liquid was recovered and subjected to ethanol treatment for causing precipitation of nucleic acid components. The precipitate was dissolved in 1 ml of TE buffe and the solution was used as a sample. Further, a 1 µg/ml solution of human placenta DNA was prepared and also subjected to PCR.

Results

As shown in Tables 55-57, the primers used did not cause amplification of any of the various DNAs, including DNAs derived from diarrhea-causing microorganisms. Therefore, it can be declared that the oligonucleotides, namely primers, of the invention react selectively with pathogenic Escherichia coli strains having the STh or STp gene.

The agarose gel electrophoresis described herein, when carried out under the conditions mentioned above, can distinguish nucleotides of 100 base pairs or less in size from each other when they differ in size by 5 to 10 base pairs, and nucleotides of 100 to 500 base pairs in size from each other when they differ in size by 10 to 20 base pairs.

The precision of nucleotide size determination can be improved by using acrylamide, for instance, as the gel and, by doing so, the reliability in the selective detection of the STh or STp gene can probably be further improved.

EXAMPLE 6

Detection of the STh gene of toxigenic Escherichia coli

Experiment 1

Sample preparation

Samples were prepared by the same technique as used in Example 5.

Primer synthesis

Based on the base sequence of the STh gene of toxigenic Escherichia coli [Moseley, S. L., et al., Infect. Immun., 39, 1167-1174 (1983)], the sequences (c), (d) and (e) shown in Claim 8 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical

synthesis and purification of the oligonucleotides synthesized were performed in the same manner as in Example 1.

PCR

The same technique as used in Example 5 was used except that the following primer combinations were used.

Primer (1)	Primer (2)
(c)	(e)
(d)	(e)

Detection

The technique used in Example 5 was used.

Results

As mentioned above, the base sequence of the STh gene of toxigenic Escherichia coli has already been determined. Therefore, the size of the nucleotide to be amplified by the PCR using the oligonucleotides, or primers, of the invention can be readily estimated.

Thus, when the primer combinations (c) + (e) and (d) + (e) are used, the nucleotides amplified are expected to have the sizes of 137 and 127 bases, respectively. When the size of the amplified nucleotide was in agreement with either of these estimated values, it was judged that each primer could contribute to correct amplification of the target region in the STh gene. This judgment was indicated by "+" in Tables 33-54. When no nucleotide amplification was noted, "-" was given in the tables. The results obtained with 492 strains of pathogenic Escherichia coli are shown in Tables 33-54.

As can be seen in Tables 33-54, each of the two primer combinations could correctly detect only those strains of pathogenic Escherichia coli that had been shown to have the STh gene by the colony hybridization technique.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for pathogenic Escherichia coli strains having the STh gene, diarrhea-causing microorganisms Other than pathogenic Escherichia coli but generally included as targets in clinical laboratory testing were tested for comparison by the same method as used in Example 1.

As shown in Tables 55-57, either of the primer combinations did not cause DNA amplification for any of various DNAs including the DNAs of the diarrhea-causing microorganisms. Therefore, it can be declared that the oligonucleotides, namely primers, of the invention react selectively only with pathogenic Escherichia coli strains having the STh gene.

EXAMPLE 7

Detection of the STp gene of toxigenic Escherichia coli

Experiment 1

Sample preparation

Samples were prepared in the same manner as in Example 5.

Primer synthesis

Based on the base sequence of the STp gene of toxigenic Escherichia coli [Moseley, S. L., et al., Infect. Immun., 39, 1167-1174 (1983)], the sequences (f), (g) and (h) shown in Claim 9 were selected, and

oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis and purification of the oligonucleotides synthesized were performed as described in Example 1.

PCR

The method of Example 5 was used except that the following primer combinations were used.

Primer (1)	Primer (2)
(f)	(h)
(g)	(h)

Detection

The method of Example 5 was used.

Results

As mentioned above, the base sequence of the STp gene of toxigenic Escherichia coli has already been determined. Accordingly, the size of the nucleotide to be amplified by the PCR using the oligonucleotides, or primers, of the invention can readily be estimated.

Thus, when the primer combinations (f) + (h) and (g) + (h) are used, nucleotides of 143 and 123 bases in size, respectively, are expected to be amplified. When the size of the amplified nucleotide was in agreement with such estimated value, it was judged that each primer could contribute to correct amplification of the target region in the STh gene. This judgment was indicated by "+" in tables 33-54. When no nucleotide amplification was noted, "-" was given. The results obtained with 492 strains of pathogenic Escherichia coli are shown in Tables 33-54.

As can be seen in Tables 33-54, each of the two primer combinations could correctly detect only those pathogenic Escherichia coli strains that had been shown to have the STp gene by the colony hybridization technique.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for pathogenic Escherichia coli strains having the STp gene, diarrhea-causing microorganisms other than pathogenic Escherichia coli but generally included as targets in clinical laboratory testing were tested for comparison by the same technique as used in Example 1.

As shown in Tables 55-57, either of the primer combinations did not cause DNA amplification for any of various DNAs, such as DNAs of diarrhea-causing microorganisms. Therefore, it can be declared that the oligonucleotides, namely primers, of the invention selectively react only with pathogenic Escherichia coli strains having the STp gene.

Tables 33 -

EXAMPLE 8

Detection of the entA gene of Staphylococcus aureus Experiment 1

Sample preparation

A total of 157 stains of Staphylococcus aureus, as shown in Tables 58-63, were used. These were food poisoning case strains isolated from patients' feces or vomits, causative foods, etc. Each strain was inoculated into brain heart infusion medium (BRL) and shake-cultured overnight at 37°C under aerobic conditions. Each culture broth was diluted with 10 mM Tris-hydrochloride buffer (pH 7.5) (TE buffer), heat-treated at 95°C for 10 minutes and then centrifuged. The supernatant was used as a sample.

Primer synthesis

Based on the base sequence of the entA gene of Staphylococcus aureus [Betley, M. J. and Mekalanos, J. J., J. Bacteriol, 170, 34-41 (1988)], the sequences (a) to (f) shown in Claim 11 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis was carried out by the β-cyanoethylphosphamidite method using a Cyclone Plus DNA synthesizer (Milligen/Bio-Research). The oligonucleotides synthesized were purified by high-performance liquid chromatography using a C18 reversed phase column.

PCR

The PCR was conducted by the same method as used in Example 4.

The following primer combinations (1) + (2) were used.

Primers (1) and (2): Each an aqueous solution of the chemically synthesized and purified product mentioned above (concentration 5 OD/ml).

Primer combinations: The above-mentioned chemically synthesized and purified products were used in the following combinations.

Primer (1)	Primer (2)
(a)	(e)
(a)	(f)
(b)	(d)
(b)	(e)
(b)	(f)
(c)	(d)
(c)	(e)
(c)	(f)

Thermostable DNA polymerase: Taq DNA polymerase (5 units/ml; Perkin Elmer Cetus).

The reaction conditions were as mentioned in Example 1.

Detection

The procedure of Example 4 was followed.

Reversed passive latex agglutination (RPLA) test

A commercial RPLA kit for detecting Staphylococcus aureus neterotoxin (SET-RPLA "Seiken", purchased from Denka Seiken) was used. Samples were prepared and tested as described in the manual attached to the kit.

However, in preparing samples to be submitted to testing, some conditions were modified so that enterotoxin could be produced in sufficient amounts. Thus, while the manual teaches that shake culture should be conducted in brain heart infusion medium or the like at 37 °C for 18-20 hours, the culture in this example was carried out in brain heart infusion medium (BRL) at 37 °C for 48 hours with shaking at 100 rpm. Each culture supernatant was submitted to the RPLA test.

Results

As mentioned above, the base sequence of the entA gene of Staphylococcus aureus has already been determined. Accordingly, the size of the nucleotide to be amplified by the PCR using each pair of the oligonucleotides, or primers, of the invention can be readily estimated.

Thus, when the combination of primers (a) and (e) is used, a nucleotide of 513 bases (or 513 base pairs) in size is expected to be amplified.

For all the primer combinations shown above, the sizes (estimated values) of the nucleotides to be amplified are summarized below.

Summary of sizes (estimated values) of amplified nucleotides				
		Primer (1)		
		(a)	(b)	(c)
Primer (2)	(d)	-	274	236
	(e)	513	390	352
	(f)	546	423	385
(in bases)				

When the size of the nucleotide amplified was in agreement with such estimated value, it was judged that each primer could contribute to correct amplification of the target region in the entA gene. This judgment was indicated by "+" in Tables 58-63. When no nucleotide amplification was noted, "-" was given.

The results obtained with 157 strains of Staphylococcus aureus are shown in Tables 58-63. As can be seen in the tables, all the primer combinations shown in the tables caused gene amplification only when the strain in question was shown to be an enterotoxin A-producing strain by the RPLA method. This indicates that the primer combinations correctly caused amplification of the entA gene and thereby correctly detected Staphylococcus aureus strains having the entA gene.

Other combinations that are not shown in the tables also gave similar test results.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for Staphylococcus aureus strains having the entA gene, the primers of the invention were investigated as to whether they were reactive with the genes of food poisoning- or diarrhea-causing microorganisms other than Staphylococcus aureus but generally included as targets in clinical laboratory testing. The procedure used was the same as shown in Experiment 1 except for the method of sample preparation.

Sample preparation

Each of the strains shown in the tables was inoculated into an appropriate enrichment medium and cultured overnight at 37°C under aerobic or anaerobic conditions. (The strains cultured under anaerobic conditions were Clostridium perfringens, Campylobacter jejuni, Bacteroides flagilis, Bacteroides vulgatus and Lactobacillus acidophilus.)

Cells were recovered from each culture broth (0.5 ml) by centrifugation and washed once with TE buffer. To these cells were added an N-acetylmuramidase in 50 mM phosphate buffer (pH 7.5) and an achromopeptidase solution to final concentrations of 50 μg/ml and 1 mg/ml, respectively. The mixture was treated at 37°C for 10 minutes for causing lysis. A phenol-chloroform mixture (mixing ratio 1:1) saturated with TE buffer was added to the lysate, followed by thorough stirring.

After centrifugation, the upper layer liquid was recovered and subjected to ethanol treatment for precipitating nucleic acid components. This precipitate was dissolved in 1 ml of TE buffer and the solution was used as a sample. Further, a 1 μg/ml solution of human placenta DNA was also prepared and submitted to PCR in the same manner.

Results

As shown in Table 64, the primers used did not cause DNA amplification for any of the various DNAs tested, including food poisoning-causing microorganisms. Therefore, it can be declared that the oligonucleotides, namely primers, of the invention selectively react only with Staphylococcus aureus strains having the entA gene. The remaining primer combinations other than those shown in Table 64 also gave similar test results.

The agarose gel electrophoresis used herein in the examples, when carried out under the conditions mentioned above, can distinguish nucleotides of 100 base pairs or less in size from each other when they differ in size by 5 to 10 base pairs, and nucleotides of 100 to 500 base pairs in size from each other when they differ in size by 10 to 20 base pairs.

Furthermore, the precision of nucleotide size determination can be improved by using acrylamide, for instance, as the gel and, by doing so, the reliability in the selective detection of the entA gene can probably be further improved.

EXAMPLE 9

Detection of the entB gene of Staphylococcus aureus

Experiment 1

Sample preparation

Samples were prepared in the same manner as in Example 8.

Primer synthesis

Based on the base sequence of the entB gene of Staphylococcus aureus [Ranelli, D. M. et al., Proc. Natl. Acad. Sci. U.S.A., 82, 5850-5854 (1985)], the sequences (g) to (l) shown in Claim 12 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis and purification of the oligonucleotides synthesized were performed in the same manner as in Example 8.

PCR

The PCR was carried out in the same manner as in Example 8 except that the following primer combinations were used.

Primer (1)	Primer (2)
(g)	(j)
(g)	(k)
(h)	(j)
(h)	(k)
(h)	(l)
(i)	(k)
(i)	(l)

Detection

The method described in Example 4 was used.

Reversed passive latex agglutination (RPLA) test

The method described in Example 8 was used.

Results

As mentioned above, the base sequence of the entB gene of Staphylococcus aureus has already been determined. Accordingly, the size of the nucleotide to be amplified by the PCR using the oligonucleotides, namely primers, of the invention can be readily estimated.

Thus, when the primer combination (g) + (j) is used, a nucleotide of 304 bases (or 304 base pairs) is expected to be amplified.

For all the primer combinations used in this example, the sizes (estimated values) of the nucleotides to be amplified are summarized below.

Summary of the sizes (estimated values) of the nucleotides to be amplified				
		Primer (1)		
		(g)	(h)	(i)
Primer (2)	(j)	304	241	-
	(h)	391	328	197
	(l)	-	419	288
(in bases)				

When the size of the nucleotide amplified was in agreement with such estimated value, it was judged that each primer contributed to correct amplification of the target region in the entB gene. This judgment was indicated by " + " in Tables 58-63. When no nucleotide amplification was noted, this fact was indicated by "-".

The results obtained with 157 strains of Staphylococcus aureus are shown in Tables 58-63. As can be seen in the tables, all the primer combinations shown caused gene amplification only for those strains that had been shown to be enterotoxin B producers by the RPLA method. Thus it is clear that said primer combinations can cause correct amplification of the entB gene and thus correctly detect Staphylococcus aureus strains having the entB gene.

The remaining combinations other than those shown in the tables also gave similar results.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for Staphylococcus aureus strains having the entB gene, the primers of the invention were examined as to whether they reacted with the genes of food poisoning- or diarrhea-causing microorganisms other than Staphylococcus aureus but generally included as targets in clinical laboratory testing were also tested by following the procedure of Example 8.

As shown in Table 64, the primers used did not cause DNA amplification for any of various DNAs, such as food poisoning-causing microorganisms. Therefore, it can be declared that the oligonucleotides, namely primers, of the invention selectively react only with Staphylococcus aureus strains having the entB gene. The remaining primer combinations other than those shown in the tables also gave similar test results.

EXAMPLE 10

Detection of the entC gene of Staphylococcus aureus

Experiment 1

Sample preparation

Samples were prepared in the same manner as in Example 8.

Primer synthesis

Based on the base sequence of the entC gene of Staphylococcus aureus [Betley, M. J. and Mekalanos, J. J., J. Bacteriol, 170, 34-41 (1988)], the base sequences (m) to (r) shown in Claim 13 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis and purification of the oligonucleotides synthesized were performed in the same manner as in Example 8.

PCR

The PCR procedure of Example 4 was followed except that the following primer combinations were used.

Primer (1)	Primer (2)
(m)	(q)
(m)	(r)
(n)	(q)
(n)	(r)
(o)	(q)
(o)	(r)
(p)	(q)
(p)	(r)

Detection

The detection procedure of Example 4 was followed.

Reversed passive latex agglutination (RPLA) test

The RPLA procedure of Example 8 was followed.

Results

As mentioned above, the base sequence of the entC gene of Staphylococcus aureus has already been determined. Therefore, the size of the nucleotide to be amplified by the PCR using the oligonucleotides, or primers, of the invention can be readily estimated.

Thus, when the combination of primers (m) and (q) is used, a nucleotide of 282 bases (or 282 base pairs) in size is expected to be amplified.

For all the primer combinations of the invention as used in this example, the sizes (estimated values) of the respective nucleotides to be amplified are shown below.

Summary of sizes (estimated values) of amplified nucleotides					
		Primer (1)			
		(m)	(n)	(o)	(p)
Primer (2)	(q)	282	274	236	99
	(r)	478	420	342	295
(in bases)					

When the size of the nucleotide amplified was in agreement with such an estimated value, it was judged that each primer contributed to correct amplification of the target region in the entC gene. This judgement is indicated by "+" in Tables 58-63, while no nucleotide amplification is indicated by "-".

The results obtained with 157 strains of Staphylococcus aureus are shown in Tables 58-63. As can be seen from the tables, all the primer combinations shown caused gene amplification only for those strains that had been shown to be enterotoxin C-producing strains by the RPLA technique. They caused correct amplification of the entC gene and correctly detected Staphylococcus aureus strains having the entC gene.

The remaining combinations other than those shown in the tables also gave similar results.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for Staphylococcus aureus strains having the entC gene, food poisoning- or diarrhea-causing microorganisms other than Staphylococcus aureus but generally includes as targets in clinical laboratory testing were studied, by the same technique as used in Example 1, as to whether the primers of the invention react with the genes of said microorganisms.

As shown in Table 64, the primer combinations used did not cause DNA amplification for any of various DNAs, including DNAs of food poisoning-causing microorganisms. Therefore, it can be declared that the

oligonucleotides, or primers, of the invention selectively react only with Staphylococcus aureus strains having the entC gene. The remaining primer combinations other than those shown in the Table also gave similar results.

EXAMPLE 11

Detection of the entD gene of Staphylococcus aureus

Experiment 1

Sample preparation

Samples were prepared in the same manner as in Example 8.

Primer synthesis

Based on the base sequence of the entD gene of Staphylococcus aureus [Betley, M. J. and Mekalanos, J. J., J. Bacteriol, 170, 34-41 (1988)], the base sequences (s) to (z) shown in Claim 14 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis and purification of the oligonucleotides synthesized were performed in the same manner as in Example 8.

PCR

The PCR procedure of Example 4 was followed except that the following primer combinations were used.

Primer (1)	Primer (2)
(s)	(w)
(s)	(y)
(s)	(z)
(t)	(w)
(t)	(x)
(t)	(z)
(u)	(x)
(u)	(y)
(v)	(y)
(v)	(z)

Detection

The technique described in Example 4 was used.

Reversed passive latex agglutination (RPLA) test

The RPLA procedure of Example 8 was followed.

As mentioned above, the base sequence of the entD gene of Staphylococcus aureus has already been determined. Therefore, the size of the nucleotide to be amplified by the PCR using the oligonucleotides, or primers, of the invention can be readily estimated.

Thus, when the combination of primers (s) and (w) is used, a nucleotide of 211 bases (or 211 base pairs) in size is expected to be amplified.

For all the primer combinations of the invention as used in this example, the sizes (estimated values) of the respective nucleotides to be amplified are shown below.

Summary of sizes (estimated values) of amplified nucleotides					
		Primer (1)			
		(s)	(t)	(u)	(v)
Primer (2)	(w)	211	142	-	-
	(x)	-	226	125	-
	(y)	474	405	304	199
	(z)	501	432	-	226
(in bases)					

When the size of the nucleotide amplified was in agreement with such an estimated value, it was judged that each primer contributed to correct amplification of the target region in the entD gene. This judgement is indicated by "+" in Tables 58-63, while no nucleotide amplification is indicated by "-".

The results obtained with 157 strains of Staphylococcus aureus are shown in Tables 58-63. As can be seen from the tables, all the primer combinations shown caused gene amplification only for those strains that had been shown to be enterotoxin D-producing strains by the RPLA technique. They caused correct amplification of the entD gene and correctly detected Staphylococcus aureus strains having the entD gene.

The remaining combinations other than those shown in the tables also gave similar results.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for Staphylococcus aureus strains having the entD gene, food poisoning- or diarrhea-causing microorganisms other than Staphylococcus aureus but generally includes as targets in clinical laboratory testing were studied, by the same technique as used in Example 1, as to whether the primers of the invention react with the genes of said microorganisms.

As shown in Table 64, the primer combinations used did not cause DNA amplification for any of various DNAs, including DNAs of food poisoning-causing microorganisms. Therefore, it can be declared that the oligonucleotides, or primers, of the invention selectively react only with Staphylococcus aureus strains having the entD gene. The remaining primer combinations other than those shown in the Table also gave similar results.

By using, in accordance with the invention, the PCR method and the primers the targets of which are genes most closely associated with pathogenicity, it is possible, in detecting bacteria having such genes, namely (1) Vibrio parahaemolyticus and Vibrio species, (2) toxigenic Escherichia coli, (3) toxigenic Escherichia coli having the STh or STp gene and (4) Staphylococcus aureus, to attain high detection sensitivity as a result of gene amplification and, at the same time, high selectivity as a result of the reaction being defined by two or more primers.

Since the detection sensitivity is high, samples are needed only in small amounts, and this makes sample pretreatment simple and easy. In the examples described herein, the reaction time was 3 hours and the time for detection procedure was as short as 30 minutes. The detection may be made easily by using a simple apparatus or instrument. Moreover, when agarose gel electrophoresis and nucleic acid staining with ethidium bromide are used for detection purposes, the detection can be carried out without labeling primers or others and, in addition, nucleic acid size determination in possible. The test results thus become highly reliable.

Vibrio parahaemolyticus is a pathogen ranking highest among food poisoning-causing bacteria in Japan. The health disturbance caused by this pathogen mostly lies in gastroenteritis, with diarrhea and abdominal pain as main symptoms. Among pathogenic factors of such gastroenteritis, thermostable direct hemolysin (TDH) is currently at the center of concern. Vibrio parahaemolyticus strains having the tdh gene, which are causative of food poisoning, can be detected selectively by taking the tdh gene coding for thermostable direct hemolysin as the target nucleotide for the primers.

Furthermore, thermostable direct hemolysin-related hemolysin (TRH), a pathogenic factor similar to TDH but essentially different therefrom, has recently been discovered and, currently, importance has been attached to the pathogenicity of TRH-producing Vibrio parahaemolyticus in food poisoning cases. In accordance with the invention, trh gene-containing Vibrio parahaemolyticus strains as food poisoning-causing organisms can be selectively detected by taking the trh gene coding for TRH as the target nucleotide to the primers.

In identifying a food poisoning-causing microorganism as pathogenic Escherichia coli, it is important to know whether the organism is capable of producing LT. Since no other living species has the ability to produce LT, pathogenic Escherichia coli can be selectively detected by taking the LT gene as the target of the primers.

Recent progress in bacteriology has revealed that organisms equally identifiable and classifiable as Escherichia coli include strains of various types, for example strains pathogenic to humans and strains nonpathogenic to humans.

For accurate identification of a factor causative of food poisoning or diarrhea as Escherichia coli, it is therefore essential to examine as to whether the Escherichia coli strain in question produces a pathogenic factor such as a toxin. In accordance with the invention, pathogenic Escherichia coli can be selectively detected in such situation by selectively detecting the gene encoding STh or STp, which is one of pathogenic factors Of Escherichia coli.

In view of recent findings in bacteriology, detection and affirmation of a strain of Staphylococcus aureus as a food poisoning- or diarrhea-causing factor can hardly be regarded as accurate and correct unless said strain has been checked as to the production of the corresponding pathogenic factor, namely enterotoxin or the like, and further, in certain cases, the type of the pathogenic factor. In accordance with the invention, Staphylococcus aureus strains causing food poisoning, diarrhea or the like can be accurately detected by detecting the enterotoxin gene, one of the pathogenic factor genes of Staphylococcus aureus.

EXAMPLE 12

Detection of the entE gene of Staphylococcus aureus Experiment 1.

Sample preparation

A total of 17 strains of Staphlylococcus aureus, as shown in Table 65, were used. Samples were prepared in the same manner as in Example 8.

The enterotoxin producing abilities of these strains were confirmed by Reversed Passive Latex Agglutination : RPLA. The ability of FRI-326 strain was derived from a literature of American Type Culture Collection.

Primer synthesis

Based on the base sequence of the entB gene of Staphylococcus aureus [Ranelli, D. M. et al., Proc. Natl. Acad. Sci. U.S.A., 82, 5850-5854 (1985)], the sequences (a) to (f) shown in Claim 15 were selected, and oligonucleotides having the same sequences as those selected were chemically synthesized. The chemical synthesis and purification of the oligonucleotides synthesized were performed in the same manner as in Example 8.

PCR

The PCR was carried out in the same manner as in Example 8 except that the following primer combinations were used.

Primer (1)	Primer (2)
(a)	(d)
(a)	(f)
(b)	(c)
(b)	(e)

Detection

The method described in Example 4 was used.

Reversed passive latex agglutination (RPLA) test

The method described in Example 8 was used.

Results

As mentioned above, the base sequence of the entE (see) gene of Staphylococcus aureus has already been determined. Accordingly, the size of the nucleotide to be amplified by the PCR using the oligonucleotides, namely primers, of the invention can be readily estimated.

Thus, when the primer combination (a) + (d) is used, a nucleotide of 481 bases (or 481 base pairs) is expected to be amplified.

For all the primer combinations used in this example, the sizes (estimated values) of the nucleotides to be amplified are summarized below.

Summary of the sizes (estimated values) of the nucleotides to be amplified

		Primer (1)	
		(a)	(b)
Primer (2)	(c)	-	292
	(d)	481	- -
	(e)	-	373
	(f)	557	-
(in bases)			

When the size of the nucleotide amplified was in agreement with such estimated value, it was judged that each primer contributed to correct amplification of the target region in the entE gene. This judgment was indicated by "+" in Table 65. When no nucleotide amplification was noted, this fact was indicated by "-".

The results obtained with 17 strains of Staphylococcus aureus are shown in Table 65. As can be seen in the tables, all the primer combinations shown caused gene amplification only for FRI-326 strain. Thus it is clear that said primer combinations can cause correct amplification of the entE gene and thus correctly detect Staphylococcus aureus strains having the entE gene.

Southern blot hybridization test was carried out by using an oligonucleotide probe having a sequence complementary to ent E (see) gene sequence. It is confirmed that the amplified DNA was derived from ent E (see) gene.

Experiment 2

To ascertain whether the results obtained in Experiment 1 were selective for Staphylococcus aureus strains having the entE gene, the primers of the invention were examined as to whether they reacted with the genes of food poisoning- or diarrhea-causing microorganisms other than Staphylococcus aureus but generally included as targets in clinical laboratory testing were also tested by following the procedure of Example 8.

As shown in Table 66, the primers used did not cause DNA amplification for any of various DNAs, such as food poisoning-causing microorganisms. Therefore, it can be declared that the oligonucleotides, namely primers, of the invention selectively react only with Staphylococcus aureus strains having the entE gene. The remaining primer combinations other than those shown in the tables also gave similar test results.

"Sequence Listing"

SEQ ID NO: 1

SEQUENCE LENGTH	:	19 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Vibrio parahaemolyticus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	GGCTCAAAATGGTTAAGCG

SEQ ID NO: 2

SEQUENCE LENGTH	:	19 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Vibrio parahaemolyticus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CATTTCCGCTCTCATATGC

SEQ ID NO: 3

SEQUENCE LENGTH	:	20 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Vibrio parahaemolyticus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CCATCTGTCCCTTTTCCTGC

SEQ ID NO: 4

SEQUENCE LENGTH	:	19 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Vibrio parahaemolyticus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CCAAATACATTTTACTTGG

SEQ ID NO: 5

SEQUENCE LENGTH: 20 bases
SEQUENCE TYPE : nucleic acid
STRANDEDNESS : single
TOPOLOGY : linear
MOLECULE TYPE : genomic DNA
HYPOTHETICAL : NO
ANTISENSE : NO
ORIGINAL SOURCE: Vibrio parahaemolyticus
FEATURE : IDENTIFICATION METHOD S
SEQUENCE : GGTACTAAATGGCTGACATC

SEQ ID NO: 6

SEQUENCE LENGTH: 20 bases
SEQUENCE TYPE : nucleic acid
STRANDEDNESS : single
TOPOLOGY : linear
MOLECULE TYPE : genomic DNA
HYPOTHETICAL : NO
ANTISENSE : NO
ORIGINAL SOURCE: Vibrio parahaemolyticus
FEATURE : IDENTIFICATION METHOD S
SEQUENCE : CCACTACCACTCTCATATGC

SEQ ID NO: 7

SEQUENCE LENGTH	:	20 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Vibrio parahaemolyticus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	GGCTCAAAATGGTTAAGCGC

SEQ ID NO: 8

SEQUENCE LENGTH	:	20 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Vibrio parahaemolyticus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	TGGCGTTTCATCCAAATACG

SEQ ID NO: 9

SEQUENCE LENGTH: 19 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Escherichia coli H10407

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : CCCAGATGAAATAAAACGT

SEQ ID NO: 10

SEQUENCE LENGTH: 19 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Escherichia coli H10407

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : CCTGAGATATATTGTGCTC

SEQ ID NO: 11

SEQUENCE LENGTH	:	22 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Escherichia coli H10407
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	ACAAACCGGCTTTGTCAGATAT

SEQ ID NO: 12

SEQUENCE LENGTH	:	22 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Escherichia coli H10407
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	GTTATATATGTCAACCTCTGAC

SEQ ID NO: 13

SEQUENCE LENGTH	:	21 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	*Escherichia coli* H10407
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	ACCGGTATTACAGAAATCTGA

SEQ ID NO: 14

SEQUENCE LENGTH	:	24 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	*Escherichia coli*
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	TGTAATTTTCTCTTTTGAAGACTC

SEQ ID NO: 15

SEQUENCE LENGTH: 22 bases
SEQUENCE TYPE : nucleic acid
STRANDEDNESS : single
TOPOLOGY : linear
MOLECULE TYPE : genomic DNA
HYPOTHETICAL : NO
ANTISENSE : NO
ORIGINAL SOURCE: Escherichia coli
FEATURE : IDENTIFICATION METHOD S
SEQUENCE : ATTACAACACAGTTCACAGCAG

SEQ ID NO: 16

SEQUENCE LENGTH: 19 bases
SEQUENCE TYPE : nucleic acid
STRANDEDNESS : single
TOPOLOGY : linear
MOLECULE TYPE : genomic DNA
HYPOTHETICAL : NO
ANTISENSE : NO
ORIGINAL SOURCE: Escherichia coli
FEATURE : IDENTIFICATION METHOD S
SEQUENCE : CCTCAGGATGCTAAACCAG

SEQ ID NO: 17

SEQUENCE LENGTH	:	20 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Escherichia coli
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	AGGATGCTAAACCAGTAGAG

SEQ ID NO: 18

SEQUENCE LENGTH	:	22 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Escherichia coli
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	AATTCACAGCAGTAATTGCTAC

SEQ ID NO: 19

SEQUENCE LENGTH	:	21 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	*Escherichia coli*
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	TCTTTCCCCTCTTTTAGTCAG

SEQ ID NO: 20

SEQUENCE LENGTH	:	21 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	*Escherichia coli*
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	GTCAACTGAATCACTTGACTC

SEQ ID NO: 21

SEQUENCE LENGTH: 21 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Escherichia coli

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : TCACAGCAGTAAAATGTGTTG

SEQ ID NO: 22

SEQUENCE LENGTH: 20 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : GTCTGAATTGCAGGGAACAG

SEQ ID NO: 23

SEQUENCE LENGTH	:	21 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CTTTTTTACAGATCATTCGTG

SEQ ID NO: 24

SEQUENCE LENGTH	:	24 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	TAGATTTTGATTCAAAGGATATTG

SEQ ID NO: 25

SEQUENCE LENGTH	:	22 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	*Staphylococcus aureus*
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CTTATTCGTTTTAACCGTTTCC

SEQ ID NO: 26

SEQUENCE LENGTH	:	20 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	*Staphylococcus aureus*
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	AACACGATTAATCCCCTCTG

SEQ ID NO: 27

SEQUENCE LENGTH: 22 bases
SEQUENCE TYPE : nucleic acid
STRANDEDNESS : single
TOPOLOGY : linear
MOLECULE TYPE : genomic DNA
HYPOTHETICAL : NO
ANTISENSE : NO
ORIGINAL SOURCE: Staphylococcus aureus
FEATURE : IDENTIFICATION METHO S
SEQUENCE : TCGTAATTAACCGAAGGTTCTG

SEQ ID NO: 28

SEQUENCE LENGTH: 24 bases
SEQUENCE TYPE : nucleic acid
STRANDEDNESS : single
TOPOLOGY : linear
MOLECULE TYPE : genomic DNA
HYPOTHETICAL : NO
ANTISENSE : NO
ORIGINAL SOURCE: Staphylococcus aureus
FEATURE : IDENTIFICATION METHOD S
SEQUENCE : AAATCTATAGATCAATTTCTATAC

SEQ ID NO: 29

SEQUENCE LENGTH: 22 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : AATTATGATAATGTTCGAGTCG

SEQ ID NO: 30

SEQUENCE LENGTH: 21 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : TTCGCATCAAACTGACAAACG

SEQ ID NO: 31

SEQUENCE LENGTH	:	21 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CATCTTCAAATACCCGAACAG

SEQ ID NO: 32

SEQUENCE LENGTH	:	20 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CCAAATAGTGACGAGTTAGG

SEQ ID NO: 33

SEQUENCE LENGTH: 22 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : TCATACCAAAAGCTATTCTCAT

SEQ ID NO: 34

SEQUENCE LENGTH: 21 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : TCTGTAGATAAATTTTTGGCA

SEQ ID NO: 35

SEQUENCE LENGTH:		24 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE:		Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	AAAATTATGACAAAGTGAAAACAG

SEQ ID NO: 36

SEQUENCE LENGTH:		23 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE:		Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	ATGGATCAAATTACTATGTAAAC

SEQ ID NO: 37

SEQUENCE LENGTH: 20 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : GTAGGTAAAGTTACAGGTGG

SEQ ID NO: 38

SEQUENCE LENGTH: 23 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : TATAAGTACATTTTGTAAGTTCC

SEQ ID NO: 39

SEQUENCE LENGTH:		22 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE:		Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	CATACCAAAAGTATTGCCGTT

SEQ ID NO: 40

SEQUENCE LENGTH:		23 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE:		Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	AAAATCTGAATTAAGTAGTACCG

SEQ ID NO: 41

SEQUENCE LENGTH: 23 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : ATAGGAGAAAATAAAAGTACAGG

SEQ ID NO: 42

SEQUENCE LENGTH: 21 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : CTTCAATTCAAAAGAAATGGC

SEQ ID NO: 43

SEQUENCE LENGTH	:	21 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	TTGTACATATGGAGGTGTCAC

SEQ ID NO: 44

SEQUENCE LENGTH	:	23 bases
SEQUENCE TYPE	:	nucleic acid
STRANDEDNESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	TTTTAGATTTGAAATGTTGAGCC

SEQ ID NO: 45

SEQUENCE LENGTH: 21 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : TGACACCTCCATATGTACAAG

SEQ ID NO: 46

SEQUENCE LENGTH: 25 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : ATTATACAATTTTAAATCCTTTTGC

SEQ ID NO: 47

SEQUENCE LENGTH: 21 bases

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : CTGTATTTTTCCTCCGAGAGT

SEQ ID NO: 48

SEQUENCE RENGTH: 24 bases

SEQUENCE TYPE : nucleic acid

STRANDENDESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : AAAAGTCTAATTACAAAGAAATG

SEQ ID NO: 49

SEQUENCE RENGTH	:	21 bases
SEQUENCE TYPE	:	nucleic acid
STRANDENDESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	GGTTTTTTCACAGGTCATCCA

SEQ ID NO: 50

SEQUENCE RENGTH	:	23 bases
SEQUENCE TYPE	:	nucleic acid
STRANDENDESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	GAACAGTTACTTCTTTTTGCTT

SEQ ID NO: 51

SEQUENCE RENGTH: 22 bases

SEQUENCE TYPE : nucleic acid

STRANDENDESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : CTGTCTGAGTTATATAAACCAA

SEQ ID NO: 52

SEQUENCE RENGTH: 20 bases

SEQUENCE TYPE : nucleic acid

STRANDENDESS : single

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

HYPOTHETICAL : NO

ANTISENSE : NO

ORIGINAL SOURCE: Staphylococcus aureus

FEATURE : IDENTIFICATION METHOD S

SEQUENCE : GCACCTTACCGCCAAAGCTG

SEQ ID NO: 53

SEQUENCE RENGTH	:	22 bases
SEQUENCE TYPE	:	nucleic acid
STRANDENDESS	:	single
TOPOLOGY	:	linear
MOLECULE TYPE	:	genomic DNA
HYPOTHETICAL	:	NO
ANTISENSE	:	NO
ORIGINAL SOURCE	:	Staphylococcus aureus
FEATURE	:	IDENTIFICATION METHOD S
SEQUENCE	:	AAACAAATCATAACTTACCGTG

Table 1

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
	V.parahaemolyticus WP-1			+		○	○	○	
1	V.parahaemolyticus	AQ 3115	-	+	-	○	○	○	-
2	V.parahaemolyticus	AQ 3170	1	+	○	○	○	○	○
3	V.parahaemolyticus	AQ 3172	2	-	○	-	-	-	-
4	V.parahaemolyticus	AQ 3178	-	-	-	-	-	-	-
5	V.parahaemolyticus	AQ 3194	1	-	○	-	-	-	○
6	V.parahaemolyticus	AQ 3214	2	-	○	-	-	-	-
7	V.parahaemolyticus	AQ 3228	2	-	○	-	-	-	-
8	V.parahaemolyticus	AQ 3230	2	-	○	-	-	-	-
9	V.parahaemolyticus	AQ 3264	-	+	-	○	○	○	-
10	V.parahaemolyticus	AQ 3295	1	+	○	○	○	○	○
11	V.parahaemolyticus	AQ 3318	-	-	-	-	-	-	-
12	V.parahaemolyticus	AQ 3326	2	-	○	-	-	-	-
13	V.parahaemolyticus	AQ 3334	-	-	-	-	-	-	-
14	V.parahaemolyticus	AQ 3336	-	-	-	-	-	-	-
15	V.parahaemolyticus	AQ 3362	1	+	○	○	○	○	○
16	V.parahaemolyticus	AQ 3367	-	-	-	-	-	-	-
17	V.parahaemolyticus	AQ 3372	2	-	○	-	-	-	-
18	V.parahaemolyticus	AQ 3392	2	-	○	-	-	-	-
19	V.parahaemolyticus	AQ 3415	-	+	-	○	○	○	-
20	V.parahaemolyticus	AQ 3421	-	-	-	-	-	-	-
21	V.parahaemolyticus	AQ 3454	1	-	○	-	-	-	○
22	V.parahaemolyticus	AQ 3458	-	-	-	-	-	-	-
23	V.parahaemolyticus	AQ 3465	1	+	○	○	○	○	○
24	V.parahaemolyticus	AQ 3491	2	-	○	-	-	-	-

Table 2

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
25	V.parahaemolyticus	AQ 3516	2	·	O	·	·	·	O
26	V.parahaemolyticus	AQ 3548	2	·	O	·	·	·	·
27	V.parahaemolyticus	AQ 3550	·	·	·	·	·	·	·
28	V.parahaemolyticus	AQ 3554	·	·	·	·	·	·	·
29	V.parahaemolyticus	AQ 3557	·	·	·	·	·	·	·
30	V.parahaemolyticus	AQ 3559	2	·	O	·	·	·	·
31	V.parahaemolyticus	AQ 3562	2	·	O	·	·	·	·
32	V.parahaemolyticus	AQ 3564	2	·	O	·	·	·	·
33	V.parahaemolyticus	AQ 3565	·	·	·	·	·	·	·
34	V.parahaemolyticus	AQ 3557	2	·	O	·	·	·	·
35	V.parahaemolyticus	AQ 3588	·	·	·	·	·	·	·
36	V.parahaemolyticus	AQ 3592	2	·	O	·	·	·	·
37	V.parahaemolyticus	AQ 3594	2	·	O	·	·	·	·
38	V.parahaemolyticus	AQ 3605	·	·	·	·	·	·	·
39	V.parahaemolyticus	AQ 3626	2	·	O	·	·	·	·
40	V.parahaemolyticus	AQ 3631	1	+	O	O	O	O	O
41	V.parahaemolyticus	AQ 3635	1	+	O	O	O	O	O
42	V.parahaemolyticus	AQ 3644	2	·	O	·	·	·	·
43	V.parahaemolyticus	AQ 3655	1	·	O	·	·	·	O
44	V.parahaemolyticus	AQ 3665	2	·	O	·	·	·	·
45	V.parahaemolyticus	AQ 3669	2	·	O	·	·	·	O
46	V.parahaemolyticus	AQ 3694	1	+	O	O	O	O	O
47	V.parahaemolyticus	AQ 3704	2	·	O	·	·	·	·
48	V.parahaemolyticus	AQ 3710	·	·	·	·	·	·	·

Table 3

No.	Strain	Stain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
49	V.parahaemolyticus	AQ 3713	1	-	○	·	·	·	○
50	V.parahaemolyticus	AQ 3727	2	-	○	·	·	·	·
51	V.parahaemolyticus	AQ 3732	1	-	○	·	·	·	○
52	V.parahaemolyticus	AQ 3739	1	+	○	○	○	○	○
53	V.parahaemolyticus	AQ 3740	-	+	·	○	○	○	·
54	V.parahaemolyticus	AQ 3741	-	+	·	○	○	○	·
55	V.parahaemolyticus	AQ 3744	1	+	○	○	○	○	○
56	V.parahaemolyticus	AQ 3754	2	-	○	·	·	·	·
57	V.parahaemolyticus	AQ 3756	-	-	·	·	·	·	·
58	V.parahaemolyticus	AQ 3765	1	+	○	○	○	○	○
59	V.parahaemolyticus	AQ 3766	1	+	○	○	○	○	○
60	V.parahaemolyticus	AQ 3776	1	+	○	○	○	○	○
61	V.parahaemolyticus	AQ 3777	2	-	○	·	·	·	·
62	V.parahaemolyticus	AQ 3785	2	-	○	·	·	·	·
63	V.parahaemolyticus	AQ 3789	2	-	○	·	·	·	·
64	V.parahaemolyticus	AQ 3794	1	-	○	·	·	·	○
65	V.parahaemolyticus	AQ 3795	1	-	○	·	·	·	○
66	V.parahaemolyticus	AQ 3801	1	+	○	○	○	○	○
67	V.parahaemolyticus	AQ 3837	2	-	○	·	·	·	·
68	V.parahaemolyticus	AQ 3838	2	-	○	·	·	·	·
69	V.parahaemolyticus	AQ 3840	2	-	○	·	·	·	·
70	V.parahaemolyticus	AQ 3853	1	+	○	○	○	○	○
71	V.parahaemolyticus	AQ 3860	1	+	○	○	○	○	○
72	V.parahaemolyticus	AQ 3869	-	-	·	·	·	·	·
73	V.parahaemolyticus	AQ 3880	2	-	○	·	·	·	·
74	V.parahaemolyticus	AQ 3881	2	-	○	·	·	·	·

Table 4

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
75	V.parahaemolyticus	AQ 3892	-	-	·	·	·	·	·
76	V.parahaemolyticus	AQ 3897	1	+	○	○	○	○	○
77	V.parahaemolyticus	AQ 3907	-	-	·	·	·	·	·
78	V.parahaemolyticus	AQ 3910	-	-	·	·	·	·	·
79	V.parahaemolyticus	AQ 3911	2	-	○	·	·	·	·
80	V.parahaemolyticus	AQ 3915	2	+	○	○	○	○	·
81	V.parahaemolyticus	AQ 3916	2	-	○	·	·	·	·
82	V.parahaemolyticus	AQ 3919	N.A.	-	○	·	·	·	·
83	V.parahaemolyticus	AQ 3924	1	+	○	○	○	○	○
84	V.parahaemolyticus	AQ 3933	-	-	·	·	·	·	·
85	V.parahaemolyticus	AQ 3945	1	-	○	·	·	·	○
86	V.parahaemolyticus	AQ 3948	-	+	·	○	○	○	·
87	V.parahaemolyticus	AQ 3951	1	-	○	·	·	·	○
88	V.parahaemolyticus	AQ 3953	-	-	·	·	·	·	·
89	V.parahaemolyticus	AQ 3966	1	+	○	○	○	○	○
90	V.parahaemolyticus	AQ 3969	2	-	○	·	·	·	·
91	V.parahaemolyticus	AQ 3980	2	-	○	·	·	·	·
92	V.parahaemolyticus	AQ 3986	-	+	·	○	○	○	·
93	V.parahaemolyticus	AQ 3531	-	+	·	○	○	○	·
94	V.parahaemolyticus	AQ 3541	-	+	·	○	○	○	·
95	V.parahaemolyticus	AQ 3551	-	+	·	○	○	○	·
96	V.parahaemolyticus	AQ 3561	-	+	·	○	○	○	·
97	V.parahaemolyticus	AQ 3571	-	+	·	○	○	○	·
98	V.parahaemolyticus	AQ 3582	-	+	·	○	○	○	·
99	V.parahaemolyticus	AQ 3591	1	-	○	·	·	·	○

Table 5

No.	Strain	Strain No.	trh	tdh	(a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
					Primer combination				
100	V.parahaemolyticus	AQ 3600	-	+	-	O	O	O	-
101	V.parahaemolyticus	AQ 3610	-	+	-	O	O	O	-
102	V.parahaemolyticus	AQ 3620	-	+	-	O	O	O	-
103	V.parahaemolyticus	AQ 3630	-	+	-	O	O	O	-
104	V.parahaemolyticus	AQ 3640	-	+	-	O	O	O	-
105	V.parahaemolyticus	AQ 3660	-	+	-	O	O	O	-
106	V.parahaemolyticus	AQ 3570	-	+	-	O	O	O	-
107	V.parahaemolyticus	AQ 3680	-	+	-	O	O	O	-
108	V.parahaemolyticus	AQ 3690	-	+	-	O	O	O	-
109	V.parahaemolyticus	AQ 3700	-	+	-	O	O	O	-
110	V.parahaemolyticus	AQ 3711	-	+	-	O	O	O	-
111	V.parahaemolyticus	AQ 3720	-	+	-	O	O	O	-
112	V.parahaemolyticus	AQ 3730	-	+	-	O	O	O	-
113	V.parahaemolyticus	AQ 3750	-	+	-	O	O	O	-
114	V.parahaemolyticus	AQ 3760	-	+	-	O	O	O	-
115	V.parahaemolyticus	AQ 3770	-	+	-	O	O	O	-
116	V.parahaemolyticus	AQ 3780	-	+	-	O	O	O	-
117	V.parahaemolyticus	AQ 3790	-	+	-	O	O	O	-
118	V.parahaemolyticus	AQ 3800	-	+	-	O	O	O	-
119	V.parahaemolyticus	AQ 3820	-	+	-	O	O	O	-
120	V.parahaemolyticus	AQ 3820	-	+	-	O	O	O	-
121	V.parahaemolyticus	AQ 3830	-	+	-	O	O	O	-
122	V.parahaemolyticus	AQ 3841	-	+	-	O	O	O	-
123	V.parahaemolyticus	AQ 3690	-	+	-	O	O	O	-
124	V.parahaemolyticus	AQ 3920	-	+	-	O	O	O	-
125	V.parahaemolyticus	AQ 3930	-	+	-	O	O	O	-

Table 6

No.	Strain	Strain No.	trh	tdh	Primer combination				
					(a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
126	V.parahaemolyticus	AQ 3940	·	·	·	·	·	·	·
127	V.parahaemolyticus	AQ 3950	·	+	·	O	O	O	·
128	V.parahaemolyticus	AQ 3960	1	·	O	·	·	·	O
129	V.parahaemolyticus	AQ 3970	·	+	·	O	O	O	·
130	V.parahaemolyticus	AQ 3981	·	+	·	O	O	O	·
131	V.parahaemolyticus	AQ 3990	·	+	·	O	O	O	·
132	V.parahaemolyticus	AQ 4000	·	+	·	O	O	O	·
133	V.parahaemolyticus	AQ 4010	·	+	·	O	O	O	·
134	V.parahaemolyticus	AQ 4030	·	+	·	O	O	O	·
135	V.parahaemolyticus	AQ 4040	·	+	·	O	O	O	·
136	V.parahaemolyticus	AQ 4050	2	+	O	O	O	O	·
137	V.parahaemolyticus	AQ 4060	·	+	·	O	O	O	·
138	V.parahaemolyticus	AQ 3128	·	+	·	O	O	O	·
139	V.parahaemolyticus	AQ 3138	·	+	·	O	O	O	·
140	V.parahaemolyticus	AQ 3158	·	+	·	O	O	O	·
141	V.parahaemolyticus	AQ 3161	·	+	·	O	O	O	·
142	V.parahaemolyticus	AQ 3171	·	+	·	O	O	O	·
143	V.parahaemolyticus	AQ 3181	1	+	O	O	O	O	O
144	V.parahaemolyticus	AQ 3202	·	+	·	O	O	O	·
145	V.parahaemolyticus	AQ 3208	·	+	·	O	O	O	·
146	V.parahaemolyticus	AQ 3211	1	+	O	O	O	O	O
147	V.parahaemolyticus	AQ 3221	·	+	·	O	O	O	·
148	V.parahaemolyticus	AQ 3231	·	+	·	O	O	O	·
149	V.parahaemolyticus	AQ 3240	·	+	·	O	O	O	·
150	V.parahaemolyticus	AQ 3241	·	+	·	O	O	O	·

Table 7

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
151	V.parahaemolyticus	AQ 3242	-	+	-	○	○	○	-
152	V.parahaemolyticus	AQ 3251	-	+	-	○	○	○	-
153	V.parahaemolyticus	AQ 3261	-	+	-	○	○	○	-
154	V.parahaemolyticus	AQ 3269	-	+	-	○	○	○	-
155	V.parahaemolyticus	AQ 3270	-	+	-	○	○	○	-
156	V.parahaemolyticus	AQ 3271	-	+	-	○	○	○	-
157	V.parahaemolyticus	AQ 3274	1	+	○	○	○	○	○
158	V.parahaemolyticus	AQ 3281	-	+	-	○	○	○	-
159	V.parahaemolyticus	AQ 3294	-	+	-	○	○	○	-
160	V.parahaemolyticus	AQ 3304	-	+	-	○	○	○	-
161	V.parahaemolyticus	AQ 3306	-	+	-	○	○	○	-
162	V.parahaemolyticus	AQ 3307	-	+	-	○	○	○	-
163	V.parahaemolyticus	AQ 3308	-	+	-	○	○	○	-
164	V.parahaemolyticus	AQ 3312	-	+	-	○	○	○	-
165	V.parahaemolyticus	AQ 3314	-	+	-	○	○	○	-
166	V.parahaemolyticus	AQ 3324	-	+	-	○	○	○	-
167	V.parahaemolyticus	AQ 3335	-	+	-	○	○	○	-
168	V.parahaemolyticus	AQ 3345	-	+	-	○	○	○	-
169	V.parahaemolyticus	AQ 3365	-	+	-	○	○	○	-
170	V.parahaemolyticus	AQ 3382	-	+	-	○	○	○	-
171	V.parahaemolyticus	AQ 3385	-	+	-	○	○	○	-
172	V.parahaemolyticus	AQ 3395	-	+	-	○	○	○	-
173	V.parahaemolyticus	AQ 3405	-	+	-	○	○	○	-
174	V.parahaemolyticus	AQ 3426	-	+	-	○	○	○	-
175	V.parahaemolyticus	AQ 3436	-	+	-	○	○	○	-

Table 8

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
176	V.parahaemolyticus	AQ 3446	-	+	-	○	○	○	-
177	V.parahaemolyticus	AQ 3451	-	+	-	○	○	○	-
178	V.parahaemolyticus	AQ 3471	-	+	-	○	○	○	-
179	V.parahaemolyticus	AQ 3492	-	+	-	○	○	○	-
180	V.parahaemolyticus	AQ 3501	-	+	-	○	○	○	-
181	V.parahaemolyticus	AQ 3511	-	+	-	○	○	○	-
182	V.parahaemolyticus	AQ 3521	-	+	-	○	○	○	-
183	V.parahaemolyticus	AQ 4070	-	+	-	○	○	○	-
184	V.parahaemolyticus	AQ 4080	-	+	-	○	○	○	-
185	V.parahaemolyticus	AQ 4090	-	+	-	○	○	○	-
186	V.parahaemolyticus	AQ 4093	1	-	○	-	-	-	○
187	V.parahaemolyticus	AQ 4095	1	-	○	-	-	-	○
188	V.parahaemolyticus	AQ 4129	1	-	○	-	-	-	○
189	V.parahaemolyticus	AQ 4100	-	+	-	○	○	○	-
190	V.parahaemolyticus	AQ 4110	-	+	-	○	○	○	-
191	V.parahaemolyticus	AQ 4120	-	+	-	○	○	○	-
192	V.parahaemolyticus	AQ 4130	-	+	-	○	○	○	-
193	V.parahaemolyticus	AQ 4133	1	-	○	-	-	-	○
194	V.parahaemolyticus	AQ 4150	-	+	-	○	○	○	-
195	V.parahaemolyticus	AQ 4160	-	+	-	○	○	○	-
196	V.parahaemolyticus	AQ 4170	-	+	-	○	○	○	-
197	V.metschnikovii	ATCC 7708	NT	-	-	-	-	-	-
198	V.cholerae O1	PB 1	-	-	-	-	-	-	-
199	V.cholerae O1	SGN 7277	-	-	-	-	-	-	-
200	V.cholerae O1	1094-79	-	-	-	-	-	-	-
201	V.cholerae O1	E 9120	-	-	-	-	-	-	-

Table 9

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(b)	(e)+(f)	(g)+(h)
202	V.cholerae O1	E 506	·	·	·	·	·	·	·
203	V.cholerae O1	PB 17	·	·	·	·	·	·	·
204	V.lumissii	ATCC 35016	·	·	·	·	·	·	·
205	V.mimicus	ATCC 33653	·	·	·	·	·	·	·
206	V.mimicus	Lab. No.1	·	·	·	○	○	○	·
207	V.mimicus	Lab. No.14	·	·	·	·	·	·	·
208	V.parahaemolyticus	AQ 4033	1	+	○	○	○	○	○
209	V.parahaemolyticus	AQ 4037	1	·	○	·	·	·	○
210	Aeromonas	Lab. No. 74	·	·	·	·	·	·	·
211	V.cholerae non O1	Lab. No. 90	·	·	·	·	·	·	·
212	V.cholerae non O1	Lab.No.91	·	+	·	○	○	○	·
213	V.cholerae non O1	Lab. No. 7	·	·	·	·	·	·	·
214	V.cholerae non O1	AQ 1254	·	·	·	·	·	·	·
215	V.cholerae non O1	AQ 1255	·	·	·	·	·	·	·
216	V.cholerae non O1	AQ 1257	·	·	·	·	·	·	·
217	V.cholerae non O1	AQ 1259	·	·	·	·	·	·	·
218	V.cholerae non O1	AQ 1261	·	·	·	·	·	·	·
219	V.cholerae non O1	AQ 1262	·	·	·	·	·	·	·
220	V.cholerae non O1	AQ 1266	·	·	·	·	·	·	·
221	V.cholerae non O1	AQ 1268	·	·	·	·	·	·	·
222	V.cholerae non O1	AQ 1271	·	·	·	·	·	·	·
223	V.cholerae non O1	AQ 1272	·	·	·	·	·	·	·
224	V.cholerae non O1	AQ 1273	·	·	·	·	·	·	·
225	V.cholerae non O1	AQ 1276	·	·	·	·	·	·	·
226	V.cholerae non O1	AQ 1278	·	·	·	·	·	·	·
227	V.cholerae non O1	AQ 1280	·	·	·	·	·	·	·

Table 10

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
228	V.cholerae non O1	AQ 1283	-	-	-	-	-	-	-
229	V.cholerae non O1	AQ 1286	-	-	-	-	-	-	-
230	V.cholerae non O1	AQ 1289	-	-	-	-	-	-	-
231	V.cholerae non O1	AQ 1290	-	-	-	-	-	-	-
232	V.cholerae non O1	AQ 1292	-	-	-	-	-	-	-
233	V.cholerae non O1	AQ 1294	-	-	-	-	-	-	-
234	V.cholerae non O1	AQ 1297	-	-	-	-	-	-	-
235	V.cholerae non O1	AQ 1299	-	-	-	-	-	-	-
236	V.cholerae non O1	AQ 1300	-	-	-	-	-	-	-
237	V.cholerae non O1	AQ 1304	-	-	-	-	-	-	-
238	V.cholerae non O1	AQ 1305	-	-	-	-	-	-	-
239	V.cholerae non O1	AQ 1306	-	-	-	-	-	-	-
240	V.cholerae non O1	AQ 1308	-	-	-	-	-	-	-
241	V.cholerae non O1	AQ 1312	-	-	-	-	-	-	-
242	V.cholerae non O1	AQ 1314	-	-	-	-	-	-	-
243	V.cholerae non O1	AQ 1315	-	-	-	-	-	-	-
244	V.cholerae non O1	AQ 1316	-	-	-	-	-	-	-
245	V.cholerae non O1	AQ 1317	-	-	-	-	-	-	-
246	V.cholerae non O1	AQ 1321	-	-	-	-	-	-	-
247	V.cholerae non O1	AQ 1322	-	-	-	-	-	-	-
248	V.cholerae non O1	AQ 1325	-	-	-	-	-	-	-
249	V.cholerae non O1	KB 274	-	-	-	-	-	-	-
250	V.cholerae non O1	KB 289	-	-	-	-	-	-	-
251	V.cholerae non O1	KB 297	-	-	-	-	-	-	-
252	V.cholerae non O1	KB 305	-	-	-	-	-	-	-

Table 11

No.	Strain	Strain No.	trh	tlh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
253	V.cholerae non O1	59H-63	·	·	·	·	·	·	·
254	V.cholerae non O1	59H-168	·	·	·	·	·	·	·
255	V.cholerae non O1	60H-113	·	·	·	·	·	·	·
256	V.fluvialis	59H-165	·	·	·	·	·	·	·
257	V.fluvialis	61H-79	·	·	·	·	·	·	·
258	V.fluvialis	61H-175	·	·	·	·	·	·	·
259	V.furnissii	61H-176	·	·	·	·	·	·	·
260	V.fluvialis	61H-178	·	·	·	·	·	·	·
261	V.furnissii	61H-180	·	·	·	·	·	·	·
262	V.furnissii	61H-212	·	·	·	·	·	·	·
263	V.mimicus	60H-39	·	+	·	○	○	○	·
264	V.cholerae O1	61H-110	·	·	·	·	·	·	·
265	V.cholerae O1	61H-151	·	·	·	·	·	·	·
266	V.cholerae O1	56H-118	·	·	·	·	·	·	·
267	V.cholerae O1	56H-119	·	·	·	·	·	·	·
268	V.fluvialis	58H-128	·	·	·	·	·	·	·
269	V.cholerae non O1	1	·	·	·	·	·	·	·
270	V.cholerae non O1	7	·	·	·	·	·	·	·
271	V.cholerae non O1	8	·	·	·	·	·	·	·
272	V.cholerae non O1	12	·	·	·	·	·	·	·
273	V.cholerae non O1	17	·	·	·	·	·	·	·
274	V.cholerae non O1	21	·	·	·	·	·	·	·
275	V.cholerae non O1	37	·	·	·	·	·	·	·
276	V.cholerae non O1	41	·	·	·	·	·	·	·
277	V.cholerae non O1	62	·	·	·	·	·	·	·

Table 12

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
278	V.cholerae non O1	70	-	-	-	-	-	-	-
279	V.cholerae non O1	75	-	-	-	-	-	-	-
280	V.cholerae non O1	102	-	-	-	-	-	-	-
281	V.cholerae non O1	106	-	-	-	-	-	-	-
282	V.cholerae non O1	107	-	-	-	-	-	-	-
283	V.cholerae non O1	109	-	-	-	-	-	-	-
284	V.cholerae non O1	111	-	-	-	-	-	-	-
285	V.cholerae non O1	126	-	-	-	-	-	-	-
286	V.cholerae non O1	128	-	-	-	-	-	-	-
287	V.cholerae non O1	129	-	-	-	-	-	-	-
288	V.cholerae non O1	131	-	-	-	-	-	-	-
289	V.cholerae non O1	133	-	-	-	-	-	-	-
290	V.cholerae non O1	138	-	-	-	-	-	-	●
291	V.cholerae non O1	139	-	-	-	-	-	-	-
292	V.cholerae non O1	142	-	-	-	-	-	-	-
293	V.cholerae non O1	147	-	-	-	-	-	-	-
294	V.cholerae non O1	150	-	-	-	-	-	-	-
295	V.cholerae non O1	151	-	-	-	-	-	-	-
296	V.cholerae non O1	157	-	-	-	-	-	-	-
297	V.cholerae non O1	184	-	-	-	-	-	-	-
298	V.parahaemolyticus	BG-50	-	-	-	-	-	-	-
299	V.parahaemolyticus	BG-51	-	-	-	-	-	-	-
300	V.parahaemolyticus	BG-52	-	-	-	-	-	-	-
301	V.parahaemolyticus	BG-53	-	-	-	-	-	-	-
302	V.parahaemolyticus	BG-56	-	-	-	-	-	-	-
303	V.parahaemolyticus	BG-57	-	-	-	-	-	-	-

Table 13

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
304	V.parahaemolyticus	BG-58	·	·	·	·	·	·	·
305	V.parahaemolyticus	BG-59	·	·	·	·	·	·	·
306	V.parahaemolyticus	BG-62	·	·	·	·	·	·	·
307	V.parahaemolyticus	BG-64	2	·	○	·	·	·	·
308	V.parahaemolyticus	BG-94	2	·	○	·	●	·	·
309	V.parahaemolyticus	BG-95	·	·	·	·	·	·	·
310	V.parahaemolyticus	BG-121	·	·	·	·	·	·	·
311	V.parahaemolyticus	BG-124	·	·	·	·	·	·	·
312	V.parahaemolyticus	BG-126	·	·	·	·	·	·	·
313	V.parahaemolyticus	BG-127	·	·	·	·	·	·	·
314	V.parahaemolyticus	BG-128	·	·	·	·	·	·	·
315	V.parahaemolyticus	BG-129	·	·	·	·	·	·	·
316	V.parahaemolyticus	BG-130	·	·	·	·	·	·	·
317	V.parahaemolyticus	BG-132	·	·	·	·	·	·	·
318	V.parahaemolyticus	BG-133	·	·	·	·	·	·	·
319	V.parahaemolyticus	BG-134	·	·	·	·	·	·	·
320	V.parahaemolyticus	BG-135	·	·	·	·	·	·	·
321	V.parahaemolyticus	BG-137	·	·	·	·	·	·	·
322	V.parahaemolyticus	BG-138	·	·	·	·	·	·	·
323	V.parahaemolyticus	BG-1	·	·	·	·	·	·	·
324	V.parahaemolyticus	BG-2	·	·	·	·	·	·	·
325	V.parahaemolyticus	BG-3	·	·	·	·	·	·	·
326	V.parahaemolyticus	BG-12	·	·	·	·	·	·	·
327	V.parahaemolyticus	BG-13	·	·	·	·	·	·	·
328	V.parahaemolyticus	BG-14	·	·	·	·	·	·	·

Table 14

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
329	V.parahaemolyticus	BG-22	·	·	·	·	·	·	·
330	V.parahaemolyticus	BG-23	·	·	·	·	·	·	·
331	V.parahaemolyticus	BG-24	·	·	·	·	·	·	·
332	V.parahaemolyticus	BG-25	·	·	·	·	·	·	·
333	V.parahaemolyticus	BG-26	·	·	·	·	·	·	·
334	V.parahaemolyticus	BG-31	·	·	·	·	·	·	·
335	V.parahaemolyticus	BG-33	·	·	·	·	·	·	·
336	V.parahaemolyticus	BG-34	·	·	·	·	·	·	·
337	V.parahaemolyticus	BG-35	·	·	·	·	·	·	·
338	V.parahaemolyticus	A-3-2	·	·	·	·	·	·	·
339	V.parahaemolyticus	AP-2	2	·	○	·	·	·	·
340	V.parahaemolyticus	AT-4	2	·	○	·	·	·	·
341	V.parahaemolyticus	AY-3-4	·	·	·	·	·	●	·
342	V.parahaemolyticus	BM-2-3	·	·	·	·	·	·	·
343	V.parahaemolyticus	CH-8-3	·	·	·	·	·	·	·
344	V.parahaemolyticus	CK-5-5	·	·	·	·	·	·	·
345	V.parahaemolyticus	CM-32-3	·	·	·	·	●	●	·
346	V.parahaemolyticus	CX-6	·	·	·	·	·	●	·
347	V.parahaemolyticus	DW-1-2	·	·	·	·	·	·	·
348	V.parahaemolyticus	EW-2-2	·	·	·	·	·	·	·
349	V.parahaemolyticus	FD-2-3	·	·	·	·	·	·	·
350	V.parahaemolyticus	FE-2-2	2	·	○	·	·	●	·
351	V.parahaemolyticus	FG-34-4	·	·	·	·	·	·	·
352	V.parahaemolyticus	GH-13-3	·	·	·	·	·	·	·
353	V.parahaemolyticus	257	1	+	○	○	○	○	○
354	V.parahaemolyticus	256	·	+	·	○	○	○	·

Table 15

No.	Strain	Strain No.	trh	tdh	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
355	V.parahaemolyticus	268	-	+	-	○	○	○	-
356	V.parahaemolyticus	269	-	+	-	○	○	○	-
357	V.parahaemolyticus	270	-	+	-	○	○	○	-
358	V.parahaemolyticus	282	-	+	-	○	○	○	-
359	V.parahaemolyticus	283	-	+	-	○	○	○	-
360	V.parahaemolyticus	284	-	+	-	○	○	○	-
361	V.parahaemolyticus	285	-	+	-	○	○	○	-
312	V.parahaemolyticus	286	-	+	-	○	○	○	-

Table 16

No.	Strain	Strain No.	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
1	Bacillus cereus	ATCC 14579	·	·	·	·	·
2	Bacillus subtilis	JCM 1465	·	·	·	·	·
3	Staphylococcus auveus	JCM 2413	·	·	·	·	·
4	Staphylococcus epidermidis	JCM 2414	·	·	·	·	·
5	Salmonella typhimurium	IFO 12529	·	·	·	·	·
6	Salmonella enteritidis	IFO 3163	·	·	·	·	·
7	Clostridium perfringens	ATCC 12917	·	·	·	·	·
8	Vibrio cholerae	ATCC 25872	·	·	·	·	·
9	Vibrio cholerae type Ogawa	ATCC 9458	·	·	·	·	·
10	Vibrio cholerae type Inaba	ATCC 9459	·	·	·	·	·
11	Vibrio fluvialis	JCM 3752	·	·	·	·	·
12	Campylobacter jejuni	JCM 2013	·	·	·	·	·
13	Campylobacter coli	JCM 2529	·	·	·	·	·
14	E. coli	JCM 1649	·	·	·	·	·
15	Yersinia enterocolitica	ATCC 9610	·	·	·	·	·
16	Sigella dysenteriae	ATCC 9361	·	·	·	·	·
17	Sigella flexneri	ATCC 29903	·	·	·	·	·
18	Sigella sonnei	ATCC 29930	·	·	·	·	·
19	Bacteroides flagilis	ATCC 23745	·	·	·	·	·
20	Bacteroides vulgatus	JCM 5826	·	○	·	·	·

Table 17

No.	Strain	Strain No.	Primer combination (a)+(b)	(c)+(d)	(e)+(d)	(e)+(f)	(g)+(h)
21	Enterococcus faecalis	JCM 5803	·	·	·	·	·
22	Klebsiella pneumoniae	JCM 1662	·	·	·	·	·
23	Proteus vulgaris	JCM 1668	·	·	·	·	·
24	Citrobacter freundii	ATCC 33128	·	·	·	·	·
25	Streptococcus pyogenes	ATCC 12344	·	·	·	·	·
26	Streptococcus pneumoniae	ATCC 33400	·	·	·	·	·
27	Haemophilus influenzae	ATCC 33391	·	·	·	·	●
28	Proteus mirabilis	ATCC 29906	·	·	·	·	·
29	Neisseria gonorrheae	ATCC 19424	·	·	·	·	·
30	Neisseria meningitidis	ATCC 13077	·	·	·	·	·
31	Listeria monocygenes	ATCC 15313	·	·	·	·	·
32	Lactobacillus acidophillus	JCM 1132	·	·	·	·	·
33	Bifidobacterium adolescentis	JCM 1275	·	·	·	·	·
34	Fusobacterium nucleatum	ATCC 25586	·	·	·	·	·
35	Propiobacterium acnes	ATCC 6919	·	·	·	·	·
36	Veillonella atypica	ATCC 17744	·	·	·	·	·
37	Pseudomonas aeruginosa	IFO 12689	·	·	·	·	·
38	Corinebacterium diphtheriae	JCM 1310	·	·	·	·	·
39	Peptostreptococcus anaerobius	ATCC 27337	·	·	·	·	·
40	Human placental DNA		·	●	·	·	·

Table 18

				Primer		
No.		Strain No.	LT gene	(a)+(b)	(c)+(d)	(e)+(d)
1	*E.coli*	WHO1	-	-	-	-
2	*E.coli*	WHO2	-	-	-	-
3	*E.coli*	WHO3	-	-	-	-
4	*E.coli*	WHO4	-	-	-	-
5	*E.coli*	WHO5	-	-	-	-
6	*E.coli*	WHO6	-	-	-	-
7	*E.coli*	WHO7	-	-	-	-
8	*E.coli*	WHO8	+	+	+	+
9	*E.coli*	WHO9	+	+	+	+
10	*E.coli*	WHO10	+	+	+	+
11	*E.coli*	WHO11	-	-	-	-
12	*E.coli*	WHO12	-	-	-	-
13	*E.coli*	WHO13	-	-	-	-
14	*E.coli*	WHO14	-	-	-	-
15	*E.coli*	WHO15	-	-	-	-
16	*E.coli*	WHO16	-	-	-	-
17	*E.coli*	WHO17	+	+	+	+
18	*E.coli*	WHO18	+	+	+	+
19	*E.coli*	WHO19	-	-	-	-
20	*E.coli*	WHO20	-	-	-	-
21	*E.coli*	WHO21	-	-	-	-
22	*E.coli*	WHO22	-	-	-	-
23	*E.coli*	WHO23	-	-	-	-
24	*E.coli*	WHO24	2+	+	+	+
25	*E.coli*	WHO25	+	+	+	+
26	*E.coli*	WHO26	w	+	+	+
27	*E.coli*	WHO27	w	+	+	+
28	*E.coli*	WHO28	+	+	+	+
29	*E.coli*	WHO29	-	-	-	-
30	*E.coli*	WHO30	-	-	-	-
31	*E.coli*	WHO31	-	-	-	-
32	*E.coli*	WHO32	-	-	-	-
33	*E.coli*	WHO33	-	-	-	-
34	*E.coli*	WHO34	-	-	-	-
35	*E.coli*	WHO35	2+	+	+	+
36	*E.coli*	WHO36	w	+	+	+
37	*E.coli*	WHO37	-	-	-	-
38	*E.coli*	WHO38	-	-	-	-
39	*E.coli*	WHO39	-	-	-	-
40	*E.coli*	WHO40	-	-	-	-

Table 19

[illegible]			LT [illegible]	[illegible]		
No.		(Strain No.)	[illegible]	(a)+(b)	(b)+(d)	(c)+(d)
41	E.coli	WHO41	-	-	-	-
42	E.coli	WHO42	-	-	-	-
43	E.coli	WHO43	+	+	+	+
44	E.coli	WHO44	+	+	+	+
45	E.coli	WHO45	+	+	+	+
46	E.coli	WHO46	-	-	-	-
47	E.coli	WHO47	-	-	-	-
48	E.coli	WHO48	-	-	-	-
49	E.coli	WHO49	-	-	-	-
50	E.coli	WHO50	-	-	-	-
51	E.coli	WHO51	-	-	-	-
52	E.coli	WHO52	-	-	-	-
53	E.coli	WHO53	-	-	-	-
54	E.coli	WHO54	-	-	-	-
55	E.coli	WHO55	-	-	-	-
56	E.coli	WHO56	-	-	-	-
57	E.coli	WHO57	+	+	+	+
58	E.coli	WHO58	+	+	+	+
59	E.coli	WHO59	-	-	-	-
60	E.coli	WHO60	-	-	-	-
61	E.coli	WHO61	-	-	-	-
62	E.coli	WHO62	-	-	-	-
63	E.coli	WHO63	+	+	+	+
64	E.coli	WHO64	-	-	-	-
65	E.coli	WHO65	-	-	-	-
66	E.coli	WHO66	-	-	-	-
67	E.coli	WHO67	+	+	+	+
68	E.coli	WHO68	2+	+	+	+
69	E.coli	WHO69	-	-	-	-
70	E.coli	WHO70	-	-	-	-
71	E.coli	WHO71	+	+	+	+
72	E.coli	WHO72	+	+	+	+
73	E.coli	WHO73	+	+	+	+
74	E.coli	WHO74	-	-	-	-
75	E.coli	WHO75	+	+	+	+
76	E.coli	WHO76	-	-	-	-
77	E.coli	WHO77	-	-	-	-
78	E.coli	WHO78	-	-	-	-
79	E.coli	WHO79	-	-	-	-
80	E.coli	WHO80	-	-	-	-

Table 20

No.		(Strain No.)	LT gene	Primer (a)+(b)	Primer (c)+(d)	Primer (e)+(d)
81	*E.coli*	WHO81	-	-	-	-
82	*E.coli*	WHO82	-	-	-	-
83	*E.coli*	WHO83	-	-	-	-
84	*E.coli*	WHO84	-	-	-	-
85	*E.coli*	WHO85	+	+	+	+
86	*E.coli*	WHO86	-	-	-	-
87	*E.coli*	WHO87	-	-	-	-
88	*E.coli*	WHO88	-	-	-	-
89	*E.coli*	WHO89	+	+	+	+
90	*E.coli*	WHO90	-	-	-	-
91	*E.coli*	WHO91	+	+	+	+
92	*E.coli*	WHO92	-	-	-	-
93	*E.coli*	WHO93	+	+	+	+
94	*E.coli*	WHO94	+	+	+	+
95	*E.coli*	WHO95	-	-	-	-
96	*E.coli*	WHO96	+	+	+	+
97	*E.coli*	WHO97	-	-	-	-
98	*E.coli*	WHO98	-	-	-	-
99	*E.coli*	WHO99	+	+	+	+
100	*E.coli*	WHO100	+	+	+	+
101	*E.coli*	WHO101	-	-	-	-
102	*E.coli*	WHO102	+	+	+	+
103	*E.coli*	WHO103	-	-	-	-
104	*E.coli*	WHO104	-	-	-	-
105	*E.coli*	WHO105	-	-	-	-
106	*E.coli*	WHO106	-	-	-	-
107	*E.coli*	WHO107	-	-	-	-
108	*E.coli*	WHO108	-	-	-	-
109	*E.coli*	WHO109	-	-	-	-
110	*E.coli*	WHO110	-	-	-	-
111	*E.coli*	WHO111	+	+	+	+
112	*E.coli*	WHO112	+	+	+	+
113	*E.coli*	WHO113	-	-	-	-
114	*E.coli*	WHO114	-	-	-	-
115	*E.coli*	WHO115	-	-	-	-
116	*E.coli*	WHO116	-	-	-	-
117	*E.coli*	WHO117	-	-	-	-
118	*E.coli*	WHO118	-	-	-	-
119	*E.coli*	WHO119	-	-	-	-
120	*E.coli*	WHO120	-	-	-	-

Table 21

				Primer		
No		(Strain No.)	LT gene	(a)+(b)	(a)+(d)	(c)+(d)
121	E.coli	WHO121	-	-	-	-
122	E.coli	WHO122	-	-	-	-
123	E.coli	WHO123	-	-	-	-
124	E.coli	WHO124	+	+	+	+
125	E.coli	WHO125	-	-	-	-
126	E.coli	WHO126	-	-	-	-
127	E.coli	WHO127	-	-	-	-
128	E.coli	WHO128	-	-	-	-
129	E.coli	WHO129	+	+	+	+
130	E.coli	WHO130	-	-	-	-
131	E.coli	WHO131	-	-	-	-
132	E.coli	WHO132	-	-	-	-
133	E.coli	WHO133	-	-	-	-
134	E.coli	WHO134	-	-	-	-
135	E.coli	WHO135	-	-	-	-
136	E.coli	WHO136	+	+	+	+
137	E.coli	WHO137	-	-	-	-
138	E.coli	WHO138	+	+	+	+
139	E.coli	WHO139	-	-	-	-
140	E.coli	WHO140	-	-	-	-
141	E.coli	WHO141	-	-	-	-
142	E.coli	WHO142	+	+	+	+
143	E.coli	WHO143	-	-	-	-
144	E.coli	WHO144	-	-	-	-
145	E.coli	WHO145	+	+	+	+
146	E.coli	WHO146	-	-	-	-
147	E.coli	WHO147	-	-	-	-
148	E.coli	WHO148	+	+	+	+
149	E.coli	WHO149	-	-	-	-
150	E.coli	WHO150	-	-	-	-
151	E.coli	WHO151	-	-	-	-
152	E.coli	WHO152	-	-	-	-
153	E.coli	WHO153	+	+	+	+
154	E.coli	WHO154	+	+	+	+
155	E.coli	WHO155	-	-	-	-
156	E.coli	WHO156	-	-	-	-
157	E.coli	WHO157	-	-	-	-
158	E.coli	WHO158	+	+	+	+
159	E.coli	WHO159	-	-	-	-
160	E.coli	WHO160	-	-	-	-

Table 22

			LT	Primer		
No.		(Strain No.)	gene	(a)+(b)	(c)+(d)	(e)+(d)
161	E. coli	WHO161	-	-	-	-
162	E. coli	WHO162	-	-	-	-
163	E. coli	WHO163	-	-	-	-
164	E. coli	WHO164	-	-	-	-
165	E. coli	WHO165	-	-	-	-
166	E. coli	WHO166	-	-	-	-
167	E. coli	WHO167	-	-	-	-
168	E. coli	WHO168	+	+	+	+
169	E. coli	WHO169	-	-	-	-
170	E. coli	WHO170	-	-	-	-
171	E. coli	WHO171	-	-	-	-
172	E. coli	WHO172	-	-	-	-
173	E. coli	WHO173	-	-	-	-
174	E. coli	WHO174	-	-	-	-
175	E. coli	WHO175	+	+	+	+
176	E. coli	WHO176	-	-	-	-
177	E. coli	WHO177	-	-	-	-
178	E. coli	WHO178	+	+	+	+
179	E. coli	WHO179	-	-	-	-
180	E. coli	WHO180	-	-	-	-
181	E. coli	WHO181	-	-	-	-
182	E. coli	WHO182	-	-	-	-
183	E. coli	WHO183	-	-	-	-
184	E. coli	WHO184	-	-	-	-
185	E. coli	WHO185	-	-	-	-
186	E. coli	WHO186	-	-	-	-
187	E. coli	WHO187	-	-	-	-
188	E. coli	WHO188	-	-	-	-
189	E. coli	WHO189	-	-	-	-
190	E. coli	WHO190	+	+	+	+
191	E. coli	WHO191	+	+	+	+
192	E. coli	WHO192	-	-	-	-
193	E. coli	WHO193	-	-	-	-
194	E. coli	WHO194	-	-	-	-
195	E. coli	WHO195	-	-	-	-
196	E. coli	WHO196	+	+	+	+
197	E. coli	WHO197	-	-	-	-
198	E. coli	WHO198	-	-	-	-
199	E. coli	WHO199	-	-	-	-
200	E. coli	WHO200	+	+	+	+

Table 23

[illegible]			LT gene	Primer		
No.		(Strain No.)		(a)+(b)	(c)+(d)	(e)+(f)
201	E.coli	21-2 1	-	-	-	-
202	E.coli	21-2 2	-	-	-	-
203	E.coli	21-2 3	-	-	-	-
204	E.coli	21-2 4	-	-	-	-
205	E.coli	7-6 2	-	-	-	-
206	E.coli	7-6 3	-	-	-	-
207	E.coli	7-6 4	-	-	-	-
208	E.coli	8-4 1	+	+	+	+
209	E.coli	8-4 3	-	-	-	-
210	E.coli	8-4 4	+	+	+	+
211	E.coli	5-1 3	-	-	-	-
212	E.coli	5-1 4	-	-	-	-
213	E.coli	7-5 3	-	-	-	-
214	E.coli	7-5 6	-	-	-	-
215	E.coli	7-5 8	-	-	-	-
216	E.coli	2-15-16 1	-	-	-	-
217	E.coli	2-15-16 3	-	-	-	-
218	E.coli	28-10 3	-	-	-	-
219	E.coli	28-10 4	-	-	-	-
220	E.coli	9-12 6	-	-	-	-
221	E.coli	9-12 7	-	-	-	-
222	E.coli	2-9-21 2	-	-	-	-
223	E.coli	2-9-21 3	-	-	-	-
224	E.coli	2-9-21 4	-	-	-	-
225	E.coli	9-21 5	-	-	-	-
226	E.coli	14-1 3	-	-	-	-
227	E.coli	14-1 4	-	-	-	-
228	E.coli	5-1 1	-	-	-	-
229	E.coli	5-1 2	-	-	-	-
230	E.coli	13-1 3	-	-	-	-
231	E.coli	13-1 5	-	-	-	-
232	E.coli	13-2 1	-	-	-	-
233	E.coli	13-2 2	-	-	-	-
234	E.coli	13-2 3	-	-	-	-
235	E.coli	13-2 4	-	-	-	-
236	E.coli	14-1 5	-	-	-	-
237	E.coli	14-1 6	-	-	-	-
238	E.coli	9-12 2	-	-	-	-
239	E.coli	9-12 5	-	-	-	-
240	E.coli	13-1 2	-	-	-	-

Table 24

			LT	Primer		
No.		(Strain No.)	gene	(a)+(b)	(c)+(d)	(e)+(d)
241	E.coli	13-1 1	-	-	-	-
242	E.coli	225-2	+	+	+	+
243	E.coli	225-3	-	-	-	-
244	E.coli	225-5	-	-	-	-
245	E.coli	229-1	-	-	-	-
246	E.coli	229-4	-	-	-	-
247	E.coli	230-2	-	-	-	-
248	E.coli	230-5	+	+	+	+
249	E.coli	232-1	-	-	-	-
250	E.coli	232-3	-	-	-	-
251	E.coli	234-1	-	-	-	-
252	E.coli	234-4	-	-	-	-
253	E.coli	235-1	-	-	-	-
254	E.coli	235-2	-	-	-	-
255	E.coli	238-1	-	-	-	-
256	E.coli	238-2	-	-	-	-
257	E.coli	238-3	-	-	-	-
258	E.coli	239-4	+	-	-	-
259	E.coli	240-3	+	+	+	+
260	E.coli	245-1	-	-	-	-
261	E.coli	245-2	+	+	+	+
262	E.coli	245-3	+	+	+	+
263	E.coli	245-4	+	+	+	+
264	E.coli	245-5	-	-	-	-
265	E.coli	252-1	+	+	+	+
266	E.coli	252-2	-	-	-	-
267	E.coli	252-3	-	-	-	-
268	E.coli	252-4	-	-	-	-
269	E.coli	252-5	-	-	-	-
270	E.coli	254-2	-	-	-	-
271	E.coli	254-5	-	-	-	-
272	E.coli	257-1	-	-	-	-
273	E.coli	257-2	-	-	-	-
274	E.coli	257-3	-	-	-	-
275	E.coli	257-4	-	-	-	-
276	E.coli	259-1	-	-	-	-
277	E.coli	259-2	-	-	-	-
278	E.coli	259-4	-	-	-	-
279	E.coli	259-5	-	-	-	-
280	E.coli	260-2	-	-	-	-

Table 25

		LT	Primer		
No.	(Strain No.)	gene	(a)+(b)	(c)+(d)	(e)+(d)
281	E.coli 260-3	+	+	+	+
282	E.coli 260-4	+	+	+	+
283	E.coli 260-5	+	+	+	+
284	E.coli 261-1	+	+	+	+
285	E.coli 261-2	-	-	-	-
286	E.coli 261-3	-	-	-	-
287	E.coli 264-1	-	-	-	-
288	E.coli 264-2	-	-	-	-
289	E.coli 264-3	-	-	-	-
290	E.coli 264-4	-	-	-	-
291	E.coli 264-5	-	-	-	-
292	E.coli 266-1	-	-	-	-
293	E.coli 266-2	-	-	-	-
294	E.coli 266-3	+	+	+	+
295	E.coli 266-4	-	-	-	-
296	E.coli 269-2	-	-	-	-
297	E.coli 281-1	-	-	-	-
298	E.coli 281-2	-	-	-	-
299	E.coli 281-3	-	-	-	-
300	E.coli 281-4	-	-	-	-
301	E.coli 281-5	-	-	-	-
302	E.coli 282-3	-	-	-	-
303	E.coli 282-5	-	-	-	-
304	E.coli 285-1	-	-	-	-
305	E.coli 285-2	-	-	-	-
306	E.coli 285-3	-	-	-	-
307	E.coli 285-4	-	-	-	-
308	E.coli 285-5	-	-	-	-
309	E.coli 286-2	-	-	-	-
310	E.coli 286-3	-	-	-	-
311	E.coli 288-1	-	-	-	-
312	E.coli 288-2	-	-	-	-
313	E.coli 288-3	+	+	+	+
314	E.coli 288-4	+	+	+	+
315	E.coli 288-5	-	-	-	-
316	E.coli 289-3	+	+	+	+
317	E.coli 292-1	-	-	-	-
318	E.coli 292-2	-	-	-	-
319	E.coli 292-5	-	-	-	-
320	E.coli 294-2	+	+	+	+

Table 26

No.		(Strain No.)	CT gene	Primer (a)+(b)	Primer (c)+(d)	Primer (e)+(d)
321	E.coli	294-3	-	-	-	-
322	E.coli	294-4	+	+	+	+
323	E.coli	294-5	-	-	-	-
324	E.coli	297-2	-	-	-	-
325	E.coli	297-3	-	-	-	-
326	E.coli	297-4	-	-	-	-
327	E.coli	297-5	-	-	-	-
328	E.coli	306-1	-	-	-	-
329	E.coli	306-3	-	-	-	-
330	E.coli	309-1	+	+	+	+
331	E.coli	309-2	+	+	+	+
332	E.coli	309-3	-	-	-	-
333	E.coli	310-2	-	-	-	-
334	E.coli	310-3	-	-	-	-
335	E.coli	310-4	-	-	-	-
336	E.coli	310-5	-	-	-	-
337	E.coli	311-1	-	-	-	-
338	E.coli	311-2	-	-	-	-
339	E.coli	311-3	+	+	+	+
340	E.coli	311-4	-	-	-	-
341	E.coli	311-5	+	+	+	+
342	E.coli	313-1	+	+	+	+
343	E.coli	313-2	-	-	-	-
344	E.coli	313-3	-	-	-	-
345	E.coli	313-4	-	-	-	-
346	E.coli	313-5	-	-	-	-
347	E.coli	322-4	+	+	+	+
348	E.coli	324-1	+	+	+	+
349	E.coli	324-2	+	+	+	+
350	E.coli	324-3	-	-	-	-
351	E.coli	324-4	+	+	+	+
352	E.coli	324-5	-	-	-	-
353	E.coli	328-1	+	+	+	+
354	E.coli	329-1	-	-	-	-
355	E.coli	329-2	-	-	-	-
356	E.coli	334-1	+	+	+	+
357	E.coli	334-2	+	+	+	+
358	E.coli	334-3	+	+	+	+
359	E.coli	334-4	+	+	+	+
360	E.coli	334-5	+	+	+	+

Table 27

			LT gene	Primer		
No.		(Strain No.)		(a)+(b)	(c)+(d)	(e)+(d)
361	*E.coli*	339-2	-	-	-	-
362	*E.coli*	339-5	-	-	-	-
363	*E.coli*	287-1	-	-	-	-
364	*E.coli*	287-2	-	-	-	-
365	*E.coli*	287-3	-	-	-	-
366	*E.coli*	287-4	-	-	-	-
367	*E.coli*	287-5	+	+	+	+
368	*E.coli*	344-1	-	-	-	-
369	*E.coli*	344-2	-	-	-	-
370	*E.coli*	344-3	-	-	-	-
371	*E.coli*	344-4	-	-	-	-
372	*E.coli*	344-5	-	-	-	-
373	*E.coli*	346-1	-	-	-	-
374	*E.coli*	346-2	-	-	-	-
375	*E.coli*	346-3	-	-	-	-
376	*E.coli*	346-4	-	-	-	-
377	*E.coli*	364-5	-	-	-	-
378	*E.coli*	348-1	+	+	+	+
379	*E.coli*	348-2	-	-	-	-
380	*E.coli*	348-3	-	-	-	-
381	*E.coli*	348-4	-	-	-	-
382	*E.coli*	348-5	-	-	-	-
383	*E.coli*	349-5	-	-	-	-
384	*E.coli*	352-2	-	-	-	-
385	*E.coli*	352-5	-	-	-	-
386	*E.coli*	357-1	+	+	+	+
387	*E.coli*	357-2	-	-	-	-
388	*E.coli*	358-1	-	-	-	-
389	*E.coli*	358-2	-	-	-	-
390	*E.coli*	358-3	-	-	-	-
391	*E.coli*	361-3	+	+	+	+
392	*E.coli*	361-4	-	-	-	-
393	*E.coli*	361-5	-	-	-	-
394	*E.coli*	366-1	-	-	-	-
395	*E.coli*	383-4	+	+	+	+
396	*E.coli*	383-5	+	+	+	+
397	*E.coli*	384-1	-	-	-	-
398	*E.coli*	385-1	+	+	+	+
399	*E.coli*	385-2	+	+	+	+
400	*E.coli*	385-3	+	+	+	+

Table 28

No.		(Strain No.)	LT gene	Primer (a)+(b)	(c)+(d)	(e)+(d)
401	*E.coli*	385-4	+	+	+	+
402	*E.coli*	385-5	+	+	+	+
403	*E.coli*	392-1	-	-	-	-
404	*E.coli*	392-2	-	-	-	-
405	*E.coli*	392-3	-	-	-	-
406	*E.coli*	392-5	-	-	-	-
407	*E.coli*	402-1	-	-	-	-
408	*E.coli*	402-2	-	-	-	-
409	*E.coli*	402-3	-	-	-	-
410	*E.coli*	402-4	-	-	-	-
411	*E.coli*	402-5	-	-	-	-
412	*E.coli*	409-2	-	-	-	-
413	*E.coli*	409-3	-	-	-	-
414	*E.coli*	409-4	-	-	-	-
415	*E.coli*	409-5	-	-	-	-
416	*E.coli*	417-1	-	-	-	-
417	*E.coli*	417-2	-	-	-	-
418	*E.coli*	417-3	-	-	-	-
419	*E.coli*	417-4	-	-	-	-
420	*E.coli*	417-5	-	-	-	-
421	*E.coli*	412-4	-	-	-	-
422	*E.coli*	421-2	-	-	-	-
423	*E.coli*	421-3	-	-	-	-
424	*E.coli*	429-3	+	+	+	+
425	*E.coli*	429-5	+	+	+	+
426	*E.coli*	430-1	+	+	+	+
427	*E.coli*	430-3	+	+	+	+
428	*E.coli*	433-1	-	-	-	-
429	*E.coli*	433-2	+	+	+	+
430	*E.coli*	433-3	W	+	W	+
431	*E.coli*	433-4	-	-	-	-
432	*E.coli*	434-1	-	-	-	-
433	*E.coli*	434-2	-	-	-	-
434	*E.coli*	434-4	+	+	+	+
435	*E.coli*	434-5	W	+	+	+
436	*E.coli*	441-1	+	+	+	+
437	*E.coli*	486-3	-	-	-	-
438	*E.coli*	486-4	-	-	-	-
439	*E.coli*	486-5	-	-	-	-
440	*E.coli*	490-2	-	-	-	-

Table 29

No.		(Strain No.)	LT gene	Primer (a)+(b)	Primer (c)+(d)	Primer (c)+(d)
441	*E.coli*	513-1	-	-	-	-
442	*E.coli*	513-2	-	-	-	-
443	*E.coli*	513-3	-	-	-	-
444	*E.coli*	513-5	-	-	-	-
445	*E.coli*	514-1	-	-	-	-
446	*E.coli*	514-2	-	-	-	-
447	*E.coli*	514-3	-	-	-	-
448	*E.coli*	514-5	-	-	-	-
449	*E.coli*	514-4	-	-	-	-
450	*E.coli*	524-2	-	-	-	-
451	*E.coli*	524-5	-	-	-	-
452	*E.coli*	530-2	-	-	-	-
453	*E.coli*	530-3	-	-	-	-
454	*E.coli*	530-4	-	-	-	-
455	*E.coli*	530-5	-	-	-	-
456	*E.coli*	531-3	-	-	-	-
457	*E.coli*	536-1	-	-	-	-
458	*E.coli*	536-2	+	+	+	+
459	*E.coli*	536-5	+	+	+	+
460	*E.coli*	554-1	-	-	-	-
461	*E.coli*	554-2	-	-	-	-
462	*E.coli*	554-3	-	-	-	-
463	*E.coli*	554-4	-	-	-	-
464	*E.coli*	554-5	-	-	-	-
465	*E.coli*	568-1	-	-	-	-
466	*E.coli*	568-2	-	-	-	-
467	*E.coli*	568-4	-	-	-	-
468	*E.coli*	568-3	-	-	-	-
469	*E.coli*	578-1	-	-	-	-
470	*E.coli*	578-2	-	-	-	-
471	*E.coli*	578-4	-	-	-	-
472	*E.coli*	578-5	-	-	-	-
473	*E.coli*	590-1	+	+	+	+
474	*E.coli*	590-2	+	+	+	+
475	*E.coli*	590-3	-	-	-	-
476	*E.coli*	590-4	+	+	+	+
477	*E.coli*	590-5	+	+	+	+
478	*E.coli*	591-1	-	-	-	-
479	*E.coli*	591-2	-	-	-	-
480	*E.coli*	591-3	-	-	-	-

Table 30

No.		(Strain No.)	LT gene	Primer (a)+(b)	Primer (c)+(d)	Primer (a)+(d)
481	*E.coli*	591-4	-	-	-	-
482	*E.coli*	592-5	-	-	-	-
483	*E.coli*	599-1	-	-	-	-
484	*E.coli*	599-2	-	-	-	-
485	*E.coli*	599-3	-	-	-	-
486	*E.coli*	599-4	-	-	-	-
487	*E.coli*	599-5	-	-	-	-
488	*E.coli*	603-4	-	-	-	-
489	*E.coli*	604-1	-	-	-	-
490	*E.coli*	604-2	-	-	-	-
491	*E.coli*	604-3	-	-	-	-
492	*E.coli*	617-1	-	-	-	-

Table 31

			Primer		
No.		(Strain No.)	(a)+(b)	(c)+(d)	(a)+(d)
1	*Bacillus cereus*	ATCC 14579	-	-	-
2	*Bacillus subtilis*	JCM 1465	-	-	-
3	*Staphylococcus auveus*	JCM 2413	-	-	-
4	*Staphylococcus epidermidis*	JCM 2414	-	-	-
5	*Salmonella typhimurium*	IFO 12529	-	-	-
6	*Salmonella enteritidis*	IFO 3163	-	-	-
7	*Clostridium perfringens*	ATCC 12917	-	-	-
8	*Vibrio cholerae*	ATCC 25872	-	-	-
9	*Vibrio cholerae type Ogawa*	ATCC 9458	-	-	-
10	*Vibrio cholerae type Inaba*	ATCC 9459	-	-	-
11	*Vibrio fluvialis*	JCM 3752	-	-	-
12	*Campylobacter jejuni*	JCM 2013	-	-	-
13	*Campylobacter coli*	JCM 2529	-	-	-
14	*E. coli*	JCM 1649	-	-	-
15	*Yersinia enterocolitica*	ATCC 9610	-	-	-
16	*Sigella dysenteriae*	ATCC 9361	-	-	-
17	*Sigella flexneri*	ATCC 29903	-	-	-
18	*Sigella sonnei*	ATCC 29930	-	-	-
19	*Bacteroides flagilis*	ATCC 23745	-	-	-
20	*Bacteroides vulgatus*	JCM 5826	-	-	-
21	*Enterococcus feacalis*	JCM 5803	-	-	-
22	*Klebsiella pneumoniae*	JCM 1662	-	-	-
23	*Proteus vulgaris*	JCM 1668	-	-	-
24	*Citrobacter freundii*	ATCC 33128	-	-	-
25	*Streptococcus pyogenes*	ATCC 12344	-	-	-
26	*Streptococcus pneumoniae*	ATCC 33400	-	-	-

Table 32

			Primer		
No.		(Strain No.)	(a)+(b)	(c)+(d)	(e)+(d)
27	*Haemophilus influenzae*	ATCC 33391	-	-	-
28	*Proteus mivabilis*	ATCC 29906	-	-	-
29	*Neisseria gonorrheae*	ATCC 19424	-	-	-
30	*Neisseria meningitidis*	ATCC 13077	-	-	-
31	*Listeria monocygenes*	ATCC 15313	-	-	-
32	*Lactobacillus acidophilus*	JCM 1132	-	-	-
33	*Bifidobacterium adolescentis*	JCM 1275	-	-	-
34	*Fusobacterium nucleatum*	ATCC 25586	-	-	-
35	*Propiobacterium acnes*	ATCC 6919	-	-	-
36	*Veillonella atypica*	ATCC 17744	-	-	-
37	*Pseudomonas aeruginosa*	IFO 12689	-	-	-
38	*Corinebacterium diphtheriae*	JCM 1310	-	-	-
39	*Peptostreptococcus anaerobi[illegible]*	ATCC 27337	-	-	-
40	*Human placental DNA*		-	-	-
41	*V.cholerae O1 ctx +*	PB 1	-	-	-
42	*V.cholerae O1 ctx +*	SGN 7277	-	-	-
43	*V.cholerae O1 ctx -*	1094-79	-	-	-
44	*V.cholerae O1 ctx +*	E 9120	-	-	-
45	*V.cholerae O1 ctx +*	E 506	-	-	-
46	*V.cholerae O1 ctx +*	PB 17	-	-	-
47	*V.cholerae O1 ctx -*	61H-110	-	-	-
48	*V.cholerae O1 ctx +*	61H-151	-	-	-
49	*V.cholerae O1 ctx -*	56H-118	-	-	-
50	*V.cholerae O1 ctx -*	56H-119	-	-	-
51	*E.coli*	HB-101	-	-	-

Table 33

			Colony hybri[illegible]		Primer combination				
No.		(Strain No.)	STh	STp	a + b	c + e	d + e	f + h	g + h
1	E.coli	WHO1	-	-	-	-	-	-	-
2	E.coli	WHO2	+	-	+	+	+	-	-
3	E.coli	WHO3	+	-	+	+	+	-	-
4	E.coli	WHO4	-	-	-	-	-	-	-
5	E.coli	WHO5	-	-	-	-	-	-	-
6	E.coli	WHO6	-	-	-	-	-	-	-
7	E.coli	WHO7	-	-	-	-	-	-	-
8	E.coli	WHO8	+	-	+	+	+	+	+
9	E.coli	WHO9	-	+	+	-	-	+	+
10	E.coli	WHO10	-	+	+	-	-	-	-
11	E.coli	WHO11	+	-	+	+	+	-	-
12	E.coli	WHO12	-	-	-	-	-	-	-
13	E.coli	WHO13	+	-	+	+	+	-	-
14	E.coli	WHO14	-	-	-	-	-	-	-
15	E.coli	WHO15	-	-	-	-	-	-	-
16	E.coli	WHO16	-	-	-	-	-	-	-
17	E.coli	WHO17	-	-	-	-	-	-	-
18	E.coli	WHO18	-	-	-	-	-	-	-
19	E.coli	WHO19	-	-	-	-	-	-	-
20	E.coli	WHO20	-	-	-	-	-	-	-
21	E.coli	WHO21	+	-	+	+	+	-	-
22	E.coli	WHO22	+	-	+	+	+	-	-

Table 34

No		(Strain No.)	Colony Hybri. STh	STp	Primer combination a + b	c + e	d + e	f + h	g + h
23	E.coli	WHO23	-	-	-	-	-	-	-
24	E.coli	WHO24	+	-	+	+	+	-	-
25	E.coli	WHO25	-	-	-	-	-	-	-
26	E.coli	WHO26	-	-	-	-	-	-	-
27	E.coli	WHO27	-	-	-	-	-	-	-
28	E.coli	WHO28	+	-	+	+	+	-	-
29	E.coli	WHO29	+	-	+	+	+	-	-
30	E.coli	WHO30	+	-	+	+	+	-	-
31	E.coli	WHO31	-	-	-	-	-	-	-
32	E.coli	WHO32	-	-	-	-	-	-	-
33	E.coli	WHO33	-	-	-	-	-	-	-
34	E.coli	WHO34	-	-	-	-	-	-	-
35	E.coli	WHO35	-	-	-	-	-	-	-
36	E.coli	WHO36	-	-	-	-	-	-	-
37	E.coli	WHO37	-	-	-	-	-	-	-
38	E.coli	WHO38	-	-	-	-	-	-	-
39	E.coli	WHO39	+	-	+	+	+	-	-
40	E.coli	WHO40	+	-	+	+	+	-	-
41	E.coli	WHO41	-	-	-	-	-	-	-
42	E.coli	WHO42	-	-	-	-	-	-	-
43	E.coli	WHO43	-	-	-	-	-	-	-
44	E.coli	WHO44	-	-	-	-	-	-	-

Table 35

No.		(Strain No.)	Colony Hybri.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
45	E.coli	WHO45	+	-	+	+	+	-	-
46	E.coli	WHO46	-	-	-	-	-	-	-
47	E.coli	WHO47	-	+	+	-	-	+	+
48	E.coli	WHO48	-	-	-	-	-	-	-
49	E.coli	WHO49	-	-	-	-	-	-	-
50	E.coli	WHO50	-	-	-	-	-	-	-
51	E.coli	WHO51	-	-	-	-	-	-	-
52	E.coli	WHO52	-	-	-	-	-	-	-
53	E.coli	WHO53	-	-	-	-	-	-	-
54	E.coli	WHO54	-	-	-	-	-	-	-
55	E.coli	WHO55	-	-	-	-	-	-	-
56	E.coli	WHO56	-	-	-	-	-	-	-
57	E.coli	WHO57	-	+	+	-	-	+	+
58	E.coli	WHO58	+	-	+	+	+	-	-
59	E.coli	WHO59	-	-	-	-	-	-	-
60	E.coli	WHO60	-	-	-	-	-	-	-
61	E.coli	WHO61	-	-	-	-	-	-	-
62	E.coli	WHO62	-	-	-	-	-	-	-
63	E.coli	WHO63	-	-	-	-	-	-	-
64	E.coli	WHO64	-	-	-	-	-	-	-
65	E.coli	WHO65	-	-	-	-	-	-	-
66	E.coli	WHO66	-	-	-	-	-	-	-

Table 36

No.		[illegible]	Colony Hybri. 5Th	Colony Hybri. 5Tp	Primer combination a + b	c + e	d + e	f + h	g + h
67	*E.coli*	WHO67	-	-	-	-	-	+	+
68	*E.coli*	WHO68	-	+	+	-	-	-	-
69	*E.coli*	WHO69	-	-	-	-	-	-	-
70	*E.coli*	WHO70	+	-	+	+	+	-	-
71	*E.coli*	WHO71	-	-	-	-	-	-	-
72	*E.coli*	WHO72	-	-	-	-	-	-	-
73	*E.coli*	WHO73	-	-	-	-	-	-	-
74	*E.coli*	WHO74	-	-	-	-	-	-	-
75	*E.coli*	WHO75	-	-	-	-	-	-	-
76	*E.coli*	WHO76	-	-	-	-	-	-	-
77	*E.coli*	WHO77	-	-	-	-	-	-	-
78	*E.coli*	WHO78	-	-	-	-	-	-	-
79	*E.coli*	WHO79	-	-	-	-	-	-	-
80	*E.coli*	WHO80	-	-	-	-	-	-	-
81	*E.coli*	WHO81	+	-	+	+	+	-	-
82	*E.coli*	WHO82	-	-	-	-	-	-	-
83	*E.coli*	WHO83	-	-	-	-	-	-	-
84	*E.coli*	WHO84	-	-	-	-	-	-	-
85	*E.coli*	WHO85	-	-	-	-	-	-	-
86	*E.coli*	WHO86	-	-	-	-	-	-	-
87	*E.coli*	WHO87	-	-	-	-	-	-	-
88	*E.coli*	WHO88	-	-	-	-	-	-	-

Table 37

			Colony Hybri.		Primer combination				
No.		(Strain No.)	STh	STp	a + b	c + e	d + e	f + h	g + h
89	E.coli	WHO89	-	-	-	-	-	-	-
90	E.coli	WHO90	-	-	-	-	-	-	-
91	E.coli	WHO91	-	-	-	-	-	-	-
92	E.coli	WHO92	-	-	-	-	-	-	-
93	E.coli	WHO93	-	-	-	-	-	-	-
94	E.coli	WHO94	-	-	-	-	-	-	-
95	E.coli	WHO95	-	-	-	-	-	-	-
96	E.coli	WHO96	-	-	-	-	-	-	-
97	E.coli	WHO97	-	-	-	-	-	-	-
98	E.coli	WHO98	-	-	-	-	-	-	-
99	E.coli	WHO99	-	-	-	-	-	-	-
100	E.coli	WHO100	-	-	-	-	-	-	-
101	E.coli	WHO101	-	-	-	-	-	-	-
102	E.coli	WHO102	-	-	-	-	-	-	-
103	E.coli	WHO103	-	-	-	-	-	-	-
104	E.coli	WHO104	-	-	-	-	-	-	-
105	E.coli	WHO105	+	-	+	+	+	-	-
106	E.coli	WHO106	-	-	-	-	-	-	-
107	E.coli	WHO107	-	-	-	-	-	-	-
108	E.coli	WHO108	-	-	-	-	-	-	-
109	E.coli	WHO109	-	-	-	-	-	-	-
110	E.coli	WHO110	-	-	-	-	-	-	-

Table 38

			Colony Hybri.		Primer combination				
No.	(Strain No.)		STh	STp	a + b	c + e	d + e	f + h	g + h
111	E.coli	WHO111	-	-	-	-	-	-	-
112	E.coli	WHO112	-	-	-	-	-	-	-
113	E.coli	WHO113	-	-	-	-	-	-	-
114	E.coli	WHO114	-	-	-	-	-	-	-
115	E.coli	WHO115	+	-	+	+	+	-	-
116	E.coli	WHO116	-	-	-	-	-	-	-
117	E.coli	WHO117	-	-	-	-	-	-	-
118	E.coli	WHO118	-	-	-	-	-	-	-
119	E.coli	WHO119	-	-	-	-	-	-	-
120	E.coli	WHO120	-	-	-	-	-	-	-
121	E.coli	WHO121	-	-	-	-	-	-	-
122	E.coli	WHO122	+	-	+	+	+	-	-
123	E.coli	WHO123	-	-	-	-	-	-	-
124	E.coli	WHO124	-	-	-	-	-	-	-
125	E.coli	WHO125	+	-	+	+	+	-	-
126	E.coli	WHO126	-	-	-	-	-	-	-
127	E.coli	WHO127	-	-	-	-	-	-	-
128	E.coli	WHO128	-	-	-	-	-	-	-
129	E.coli	WHO129	-	-	-	-	-	-	-
130	E.coli	WHO130	+	-	+	+	+	-	-
131	E.coli	WHO131	-	-	-	-	-	-	-
132	E.coli	WHO132	+	-	+	+	+	-	-

Table 39

No.		(Strain No.)	Colony Hybri.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
133	E.coli	WHO133	-	-	-	-	-	-	-
134	E.coli	WHO134	+	-	+	+	+	-	-
135	E.coli	WHO135	-	-	-	-	-	-	-
136	E.coli	WHO136	-	-	-	-	-	-	-
137	E.coli	WHO137	-	-	-	-	-	-	-
138	E.coli	WHO138	-	-	-	-	-	-	-
139	E.coli	WHO139	-	-	-	-	-	-	-
140	E.coli	WHO140	-	-	-	-	-	-	-
141	E.coli	WHO141	-	-	-	-	-	-	-
142	E.coli	WHO142	-	+	+	-	-	+	+
143	E.coli	WHO143	-	-	-	-	-	-	-
144	E.coli	WHO144	-	-	-	-	-	-	-
145	E.coli	WHO145	-	-	-	-	-	-	-
146	E.coli	WHO146	-	-	-	-	-	-	-
147	E.coli	WHO147	-	-	-	-	-	-	-
148	E.coli	WHO148	-	-	-	-	-	-	-
149	E.coli	WHO149	-	-	-	-	-	-	-
150	E.coli	WHO150	-	-	-	-	-	-	-
151	E.coli	WHO151	-	-	-	-	-	-	-
152	E.coli	WHO152	-	-	-	-	-	-	-
153	E.coli	WHO153	+	-	+	+	+	-	-
154	E.coli	WHO154	-	-	-	-	-	-	-

Table 40

			Colony Hybri.		Primer combination				
No		(Strain No.)	STh	STp	a + b	c + e	d + e	f + h	g + h
165	E.coli	WHO155	-	-	-	-	-	-	-
156	E.coli	WHO156	-	-	-	-	-	-	-
157	E.coli	WHO157	-	-	-	-	-	-	-
168	E.coli	WHO158	-	-	-	-	-	-	-
159	E.coli	WHO159	-	-	-	-	-	-	-
160	E.coli	WHO160	+	-	+	+	+	-	-
161	E.coli	WHO161	-	-	-	-	-	-	-
162	E.coli	WHO162	-	-	-	-	-	-	-
163	E.coli	WHO163	-	-	-	-	-	-	-
164	E.coli	WHO164	+	-	+	+	+	-	-
165	E.coli	WHO165	-	-	-	-	-	-	-
166	E.coli	WHO166	-	-	-	-	-	-	-
167	E.coli	WHO167	-	-	-	-	-	-	-
168	E.coli	WHO168	-	+	+	-	-	+	+
169	E.coli	WHO169	-	-	-	-	-	-	-
170	E.coli	WHO170	-	-	-	-	-	-	-
171	E.coli	WHO171	-	-	-	-	-	-	-
172	E.coli	WHO172	-	-	-	-	-	-	-
173	E.coli	WHO173	-	-	-	-	-	-	-
174	E.coli	WHO174	-	-	-	-	-	-	-
175	E.coli	WHO175	-	-	-	-	-	-	-
176	E.coli	WHO176	-	-	-	-	-	-	-

Table 41

No.	(Strain No.)		Colony Hybri.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
177	E.coli	WHO177	-	-	-	-	-	-	-
178	E.coli	WHO178	-	+	+	-	-	+	+
179	E.coli	WHO179	-	-	-	-	-	-	-
180	E.coli	WHO180	-	-	-	-	-	-	-
181	E.coli	WHO181	-	-	-	-	-	-	-
182	E.coli	WHO182	-	-	-	-	-	-	-
183	E.coli	WHO183	+	-	+	+	+	-	-
184	E.coli	WHO184	-	-	-	-	-	-	-
185	E.coli	WHO185	W	-	+	+	+	-	-
186	E.coli	WHO186	+	-	+	+	+	-	-
187	E.coli	WHO187	-	-	-	-	-	-	-
188	E.coli	WHO188	-	-	-	-	-	-	-
189	E.coli	WHO189	-	-	-	-	-	-	-
190	E.coli	WHO190	+	-	+	+	+	-	-
191	E.coli	WHO191	-	+	+	-	-	+	+
192	E.coli	WHO192	-	-	-	-	-	-	-
193	E.coli	WHO193	-	-	-	-	-	-	-
194	E.coli	WHO194	-	-	-	-	-	-	-
195	E.coli	WHO195	-	-	-	-	-	-	-
196	E.coli	WHO196	-	+	+	-	-	-	-
197	E.coli	WHO197	-	-	-	-	-	-	-
198	E.coli	WHO198	-	-	-	-	-	-	-

Table 42

No.		(Strain No.)	Colony Hybri. STh	Colony Hybri. STp	Primer combination a + b	c + e	d + e	f + h	g + h
199	E.coli	WHO199	-	-	-	-	-	-	-
200	E.coli	WHO200	-	-	-	-	-	-	-
201	E.coli	21-2 1	+	-	+	+	+	-	-
202	E.coli	21-2 2	+	-	+	+	+	-	-
203	E.coli	21-2 3	+	-	+	+	+	-	-
204	E.coli	21-2 4	+	-	+	+	+	-	-
205	E.coli	7-6 2	-	-	-	-	-	-	-
206	E.coli	7-6 3	-	-	-	-	-	-	-
207	E.coli	7-6 4	-	-	-	-	-	-	-
208	E.coli	8-4 1	+	-	+	+	+	-	-
209	E.coli	8-4 3	-	-	-	-	-	-	-
210	E.coli	8-4 4	+	-	+	+	+	-	-
211	E.coli	5-1 3	-	-	-	-	-	-	-
212	E.coli	5-1 4	-	-	-	-	-	-	-
213	E.coli	7-5 3	-	-	-	-	-	-	-
214	E.coli	7-5 6	-	-	-	-	-	-	-
215	E.coli	7-5 8	-	-	-	-	-	-	-
216	E.coli	2-15-16 1	-	-	-	-	-	-	-
217	E.coli	2-15-16 3	-	-	-	-	-	-	-
218	E.coli	28-10 3	-	-	-	-	-	-	-
219	E.coli	28-10 4	-	-	-	-	-	-	-
220	E.coli	9-12 6	-	-	-	-	-	-	-

Table 43

No.		(Strain No.)	Colony Hybri.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
221	E.coli	19-12 7	-	-	-	-	-	-	-
222	E.coli	2-9-21 2	-	-	-	-	-	-	-
223	E.coli	2-9-21 3	-	-	-	-	-	-	-
224	E.coli	2-9-21 4	-	-	-	-	-	-	-
225	E.coli	9-21 5	-	-	-	-	-	-	-
226	E.coli	14-1 3	-	-	-	-	-	-	-
227	E.coli	14-1 4	-	-	-	-	-	-	-
228	E.coli	15-1 1	-	-	-	-	-	-	-
229	E.coli	5-1 2	-	-	-	-	-	-	-
230	E.coli	13-1 3	-	-	-	-	-	-	-
231	E.coli	13-1 5	-	-	-	-	-	-	-
232	E.coli	13-2 1	-	-	-	-	-	-	-
233	E.coli	13-2 2	-	-	-	-	-	-	-
234	E.coli	13-2 3	-	-	-	-	-	-	-
235	E.coli	13-2 4	-	-	-	-	-	-	-
236	E.coli	14-1 5	-	-	-	-	-	-	-
237	E.coli	14-1 6	-	-	-	-	-	-	-
238	E.coli	9-12 2	-	-	-	-	-	-	-
239	E.coli	9-12 5	-	-	-	-	-	-	-
240	E.coli	13-1 2	-	-	-	-	-	-	-
241	E.coli	13-1 1	-	-	-	-	-	-	-
242	E.coli	225-2	+	-	+	+	+	-	-

Table 44

No.	(Strain No.)		Colony Hybr. STh	Colony Hybr. STp	Primer combination a + b	c + e	d + e	f + h	g + h
243	E.coli	225-3	-	-	-	-	-	-	-
244	E.coli	225-5	+	-	+	+	+	-	-
245	E.coli	229-1	-	-	-	-	-	-	-
246	E.coli	229-4	-	-	-	-	-	-	-
247	E.coli	230-2	-	-	-	-	-	-	-
248	E.coli	230-5	-	-	-	-	-	-	-
249	E.coli	232-1	-	-	-	-	-	-	-
250	E.coli	232-3	-	-	-	-	-	-	-
251	E.coli	234-1	-	-	-	-	-	-	-
252	E.coli	234-4	-	-	-	-	-	-	-
253	E.coli	235-1	+	-	+	+	+	-	-
254	E.coli	235-2	+	-	+	+	+	-	-
255	E.coli	238-1	-	-	-	-	-	-	-
256	E.coli	238-2	-	-	-	-	-	-	-
257	E.coli	238-3	-	-	-	-	-	-	-
258	E.coli	238-4	-	-	-	-	-	-	-
259	E.coli	240-3	-	-	-	-	-	-	-
260	E.coli	245-1	-	-	-	-	-	-	-
261	E.coli	245-2	+	-	+	+	+	-	-
262	E.coli	245-3	-	-	-	-	-	-	-
263	E.coli	245-4	+	-	+	+	+	-	-
264	E.coli	245-5	-	-	-	-	-	-	-

Table 45

No.		(Strain No.)	Colony Hybri. STh	STp	Primer combination a+b	c+e	d+e	f+h	g+h
265	E.coli	252-1	+	-	+	+	+	-	-
266	E.coli	252-2	-	-	-	-	-	-	-
267	E.coli	252-3	-	-	-	-	-	-	-
268	E.coli	252-4	-	-	-	-	-	-	-
269	E.coli	252-5	-	-	-	-	-	-	-
270	E.coli	254-2	-	-	-	-	-	-	-
271	E.coli	254-5	-	-	-	-	-	-	-
272	E.coli	257-1	-	-	-	-	-	-	-
273	E.coli	257-2	-	-	-	-	-	-	-
274	E.coli	257-3	-	-	-	-	-	-	-
275	E.coli	257-4	-	-	-	-	-	-	-
276	E.coli	259-1	-	-	-	-	-	-	-
277	E.coli	259-2	-	-	-	-	-	-	-
278	E.coli	259-4	-	-	-	-	-	-	-
279	E.coli	259-5	-	-	-	-	-	-	-
280	E.coli	260-2	-	-	-	-	-	-	-
281	E.coli	260-3	-	-	-	-	-	-	-
282	E.coli	260-4	-	-	-	-	-	-	-
283	E.coli	260-5	-	-	-	-	-	-	-
284	E.coli	261-1	-	+	+	-	-	+	+
285	E.coli	261-2	-	-	-	-	-	-	-
286	E.coli	261-3	-	-	-	-	-	-	-

Table 46

			Colony Hybri.		Primer combination				
No.		(Strain No.)	STh	STp	a + b	c + e	d + e	f + h	g + h
287	E.coli	264-1	+	-	+	+	+	-	-
288	E.coli	264-2	+	-	+	+	+	-	-
289	E.coli	264-3	+	-	+	+	+	-	-
290	E.coli	264-4	+	-	+	+	+	-	-
291	E.coli	264-5	+	-	+	+	+	-	-
292	E.coli	266-1	+	-	+	+	+	-	-
293	E.coli	266-2	+	-	+	+	+	-	-
294	E.coli	266-3	-	+	+	-	-	+	+
295	E.coli	266-4	+	-	+	+	+	-	-
296	E.coli	269-2	-	-	-	-	-	-	-
297	E.coli	281-1	-	-	-	-	-	-	-
298	E.coli	281-2	+	-	+	+	+	-	-
299	E.coli	281-3	+	-	+	+	+	-	-
300	E.coli	281-4	+	-	+	+	+	-	-
301	E.coli	281-5	+	-	+	+	+	-	-
302	E.coli	282-3	+	-	+	+	+	-	-
303	E.coli	282-5	+	-	+	+	+	-	-
304	E.coli	285-1	-	-	-	-	-	-	-
305	E.coli	285-2	-	-	-	-	-	-	-
306	E.coli	285-3	-	-	-	-	-	-	-
307	E.coli	285-4	-	-	-	-	-	-	-
308	E.coli	285-5	-	-	-	-	-	-	-

Table 47

No		(Strain No.)	Colony Hybr.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
309	E.coli	286-2	-	-	-	-	-	-	-
310	E.coli	286-3	-	-	-	-	-	-	-
311	E.coli	288-1	-	-	-	-	-	-	-
312	E.coli	288-2	-	-	-	-	-	-	-
313	E.coli	288-3	-	-	-	-	-	-	-
314	E.coli	288-4	+	-	+	+	+	-	-
315	E.coli	288-5	-	-	-	-	-	-	-
316	E.coli	289-3	-	+	+	-	-	+	+
317	E.coli	292-1	-	-	-	-	-	-	-
318	E.coli	292-2	-	-	-	-	-	-	-
319	E.coli	292-5	-	-	-	-	-	-	-
320	E.coli	294-2	-	-	-	-	-	-	-
321	E.coli	294-3	-	-	-	-	-	-	-
322	E.coli	294-4	-	-	-	-	-	-	-
323	E.coli	294-5	-	-	-	-	-	-	-
324	E.coli	297-2	-	-	-	-	-	-	-
325	E.coli	297-3	-	-	-	-	-	-	-
326	E.coli	297-4	-	-	-	-	-	-	-
327	E.coli	297-5	-	-	-	-	-	-	-
328	E.coli	306-1	-	-	-	-	-	-	-
329	E.coli	306-3	-	-	-	-	-	-	-
330	E.coli	309-1	-	+	+	-	-	+	+

Table 48

No.		(Strain No.)	Colony Hybri.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
331	E.coli	309-2	-	+	+	-	-	+	+
332	E.coli	309-3	-	-	-	-	-	-	-
333	E.coli	310-2	-	-	-	-	-	-	-
334	E.coli	310-3	-	-	-	-	-	-	-
335	E.coli	310-4	-	-	-	-	-	-	-
336	E.coli	310-5	-	-	-	-	-	-	-
337	E.coli	311-1	-	-	-	-	-	-	-
338	E.coli	311-2	-	-	-	-	-	-	-
339	E.coli	311-3	-	-	-	-	-	-	-
340	E.coli	311-4	-	-	-	-	-	-	-
341	E.coli	311-5	-	-	-	-	-	-	-
342	E.coli	313-1	-	+	+	-	-	+	+
343	E.coli	313-2	-	-	-	-	-	-	-
344	E.coli	313-3	-	-	-	-	-	-	-
345	E.coli	313-4	-	-	-	-	-	-	-
346	E.coli	313-5	-	-	-	-	-	-	-
347	E.coli	322-4	-	-	-	-	-	-	-
348	E.coli	324-1	+	-	+	+	+	-	-
349	E.coli	324-2	-	-	-	-	-	-	-
350	E.coli	324-3	-	-	-	-	-	-	-
351	E.coli	324-4	+	-	+	+	+	-	-
352	E.coli	324-5	-	-	-	-	-	-	-

Table 49

			Colony hybr.		Primer combination				
No.		(Strain No.)	STh	STp	a + b	c + e	d + e	f + h	g + h
353	E.coli	328-1	-	-	-	-	-	-	-
354	E.coli	329-1	-	-	-	-	-	-	-
355	E.coli	329-2	+	-	+	+	+	-	-
356	E.coli	334-1	-	+	+	-	-	+	+
357	E.coli	334-2	-	+	+	-	-	+	+
358	E.coli	334-3	-	+	+	-	-	+	+
359	E.coli	334-4	-	+	+	-	-	+	+
360	E.coli	334-5	-	+	+	-	-	+	+
361	E.coli	339-2	-	-	-	-	-	-	-
362	E.coli	339-5	-	-	-	-	-	-	-
363	E.coli	287-1	-	-	-	-	-	-	-
364	E.coli	287-2	-	-	-	-	-	-	-
365	E.coli	287-3	-	-	-	-	-	-	-
366	E.coli	287-4	-	-	-	-	-	-	-
367	E.coli	287-5	+	-	+	+	+	-	-
368	E.coli	344-1	-	-	-	-	-	-	-
369	E.coli	344-2	-	-	-	-	-	-	-
370	E.coli	344-3	-	-	-	-	-	-	-
371	E.coli	344-4	-	-	-	-	-	-	-
372	E.coli	344-5	-	-	-	-	-	-	-
373	E.coli	346-1	-	-	-	-	-	-	-
374	E.coli	346-2	-	-	-	-	-	-	-

Table 50

No.		(Strain No.)	Colony Hybri.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
375	E.coli	346-3	-	-	-	-	-	-	-
376	E.coli	346-4	-	-	-	-	-	-	-
377	E.coli	364-5	-	-	-	-	-	-	-
378	E.coli	348-1	-	-	-	-	-	-	-
379	E.coli	348-2	-	-	-	-	-	-	-
380	E.coli	348-3	-	-	-	-	-	-	-
381	E.coli	348-4	-	-	-	-	-	-	-
382	E.coli	348-5	-	-	-	-	-	-	-
383	E.coli	349-5	+	-	+	+	+	-	-
384	E.coli	352-2	-	-	-	-	-	-	-
385	E.coli	352-5	-	-	-	-	-	-	-
386	E.coli	357-1	+	-	+	+	+	-	-
387	E.coli	357-2	-	-	-	-	-	-	-
388	E.coli	358-1	-	-	-	-	-	-	-
389	E.coli	358-2	-	-	-	-	-	-	-
390	E.coli	358-3	-	-	-	-	-	-	-
391	E.coli	361-3	+	-	+	+	+	-	-
392	E.coli	361-4	-	-	-	-	-	-	-
393	E.coli	361-5	-	-	-	-	-	-	-
394	E.coli	366-1	+	-	+	+	+	-	-
395	E.coli	383-4	-	-	-	-	-	-	-
396	E.coli	383-5	-	-	-	-	-	-	-

Table 51

No.		(Strain No.)	Colony lysis STh	Colony lysis STp	Primer combination a + b	c + e	d + e	f + h	g + h
397	*E.coli*	384-1	-	-	-	-	-	-	-
398	*E.coli*	385-1	-	-	-	-	-	-	-
399	*E.coli*	385-2	-	-	-	-	-	-	-
400	*E.coli*	385-3	-	-	-	-	-	-	-
401	*E.coli*	385-4	-	-	-	-	-	-	-
402	*E.coli*	385-5	-	-	-	-	-	-	-
403	*E.coli*	392-1	-	-	-	-	-	-	-
404	*E.coli*	392-2	-	-	-	-	-	-	-
405	*E.coli*	392-3	-	-	-	-	-	-	-
406	*E.coli*	392-5	-	-	-	-	-	-	-
407	*E.coli*	402-1	-	-	-	-	-	-	-
408	*E.coli*	402-2	-	-	-	-	-	-	-
409	*E.coli*	402-3	-	-	-	-	-	-	-
410	*E.coli*	402-4	-	-	-	-	-	-	-
411	*E.coli*	402-5	-	-	-	-	-	-	-
412	*E.coli*	409-2	+	-	+	+	+	-	-
413	*E.coli*	409-3	+	-	+	+	+	-	-
414	*E.coli*	409-4	+	-	+	+	+	-	-
415	*E.coli*	409-5	+	-	+	+	+	-	-
416	*E.coli*	417-1	-	-	-	-	-	-	-
417	*E.coli*	417-2	-	-	-	-	-	-	-
418	*E.coli*	417-3	-	-	-	-	-	-	-

Table 52

No.	(Strain)		Colony Hybri. STh	Colony Hybri. STp	Primer combination a+b	c+e	d+e	f+h	g+h
419	*E.coli*	417-4	+	-	+	+	+	-	-
420	*E.coli*	417-5	+	-	+	+	+	-	-
421	*E.coli*	412-4	+	-	+	+	+	-	-
422	*E.coli*	421-2	+	-	+	+	+	-	-
423	*E.coli*	421-3	+	-	+	+	+	-	-
424	*E.coli*	429-3	-	-	-	-	-	-	-
425	*E.coli*	429-5	-	-	-	-	-	-	-
426	*E.coli*	430-1	-	-	-	-	-	-	-
427	*E.coli*	430-3	-	-	-	-	-	-	-
428	*E.coli*	433-1	-	+	+	-	-	+	+
429	*E.coli*	433-2	-	+	+	-	-	+	+
430	*E.coli*	433-3	-	-	-	-	-	-	-
431	*E.coli*	433-4	-	-	-	-	-	-	-
432	*E.coli*	434-1	-	-	-	-	-	-	-
433	*E.coli*	434-2	-	-	-	-	-	-	-
434	*E.coli*	434-4	-	-	-	-	-	-	-
435	*E.coli*	434-5	-	-	-	-	-	-	-
436	*E.coli*	441-1	-	-	-	-	-	-	-
437	*E.coli*	486-3	-	-	-	-	-	-	-
438	*E.coli*	486-4	-	-	-	-	-	-	-
439	*E.coli*	486-5	-	-	-	-	-	-	-
440	*E.coli*	400-2	+	-	+	+	+	-	-

Table 53

			Colony Hybri.		Primer combination				
No.		(Strain No.)	STh	STp	a + b	c + e	d + e	f + h	g + h
441	E.coli	513-1	+	-	+	+	+	-	-
442	E.coli	513-2	+	-	+	+	+	-	-
443	E.coli	513-3	-	-	-	-	-	-	-
444	E.coli	513-5	+	-	+	+	+	-	-
445	E.coli	514-1	+	-	+	+	+	-	-
446	E.coli	514-2	-	-	-	-	-	-	-
447	E.coli	514-3	+	-	+	+	+	-	-
448	E.coli	514-5	+	-	+	+	+	-	-
449	E.coli	514-4	+	-	+	+	+	-	-
450	E.coli	524-2	+	-	+	+	+	-	-
451	E.coli	524-5	-	-	-	-	-	-	-
452	E.coli	530-2	-	-	-	-	-	-	-
453	E.coli	530-3	-	-	-	-	-	-	-
454	E.coli	530-4	-	-	-	-	-	-	-
455	E.coli	530-5	-	-	-	-	-	-	-
456	E.coli	531-3	-	-	-	-	-	-	-
457	E.coli	536-1	-	-	-	-	-	-	-
458	E.coli	536-2	-	-	-	-	-	-	-
459	E.coli	536-5	-	-	-	-	-	-	-
460	E.coli	554-1	-	-	-	-	-	-	-
461	E.coli	554-2	-	-	-	-	-	-	-
462	E.coli	554-3	-	-	-	-	-	-	-

Table 54

No.	(Strain No.)		Colony Hybri.		Primer combination				
			STh	STp	a + b	c + e	d + e	f + h	g + h
463	E.coli	554-4	-	-	-	-	-	-	-
464	E.coli	554-5	-	-	-	-	-	-	-
465	E.coli	568-1	-	-	-	-	-	-	-
466	E.coli	568-2	+	-	+	+	+	-	-
467	E.coli	568-4	-	-	-	-	-	-	-
468	E.coli	568-3	-	-	-	-	-	-	-
469	E.coli	578-1	+	-	+	+	+	-	-
470	E.coli	578-2	-	-	-	-	-	-	-
471	E.coli	578-4	+	-	+	+	+	-	-
472	E.coli	578-5	-	-	-	-	-	-	-
473	E.coli	590-1	-	-	-	-	-	-	-
474	E.coli	590-2	-	-	-	-	-	-	-
475	E.coli	590-3	-	-	-	-	-	-	-
476	E.coli	590-4	-	-	-	-	-	-	-
477	E.coli	590-5	-	-	-	-	-	-	-
478	E.coli	591-1	-	-	-	-	-	-	-
479	E.coli	591-2	-	-	-	-	-	-	-
480	E.coli	591-3	-	-	-	-	-	-	-
481	E.coli	591-4	-	-	-	-	-	-	-
482	E.coli	592-5	-	-	-	-	-	-	-
483	E.coli	599-1	-	-	-	-	-	-	-
484	E.coli	599-2	-	-	-	-	-	-	-

Table 55

No.	Name		STh			STp		
			a+b	c+e	d+e	a+b	f+h	g+h
1	*Bacillus cereus*	ATCC 14579	·	·	·	·	·	·
2	*Bacillus subtilis*	JCM 1465	·	·	·	·	·	·
3	*Staphylococcus aureus*	JCM 2413	·	·	·	·	·	·
4	*Staphylococcus epidermidis*	JCM 2414	·	·	·	·	·	·
5	*Salmonella typhimurium*	IFO 12529	·	·	·	·	·	·
6	*Salmonella enteritidis*	IFO 3183	·	·	·	·	·	·
7	*Clostridium perfringens*	ATCC 12917	·	·	·	·	·	·
8	*Vibrio cholerae*	ATCC 25872	·	·	·	·	·	·
9	*Vibrio cholerae type ogawa*	ATCC 9458	·	·	·	·	·	·
10	*Vibrio cholerae type Inaba*	ATCC 9459	·	·	·	·	·	·
11	*Vibrio fluvialis*	JCM 3752	·	·	·	·	·	·
12	*Campylobacter jejuni*	JCM 2013	·	·	·	·	·	·
13	*Campylobacter coli*	JCM 2529	·	·	·	·	·	·
14	*Escherichia coli*	JCM 1649	·	·	·	·	·	·
15	*Yersinia enterocolitica*	ATCC 9610	·	·	·	·	·	·
16	*Shigella dysenteriae*	ATCC 9361	·	·	·	·	·	·
17	*Shigella flexneri*	ATCC 29903	·	·	·	·	·	·
18	*Shigella sonnei*	ATCC 29930	·	·	·	·	·	·
19	*Bacteroides fragilis*	ATCC 23745	·	·	·	·	·	·
20	*Bacteroides vulgatus*	JCM 5826	·	·	·	·	·	·

Table 56

No.	Name		STh			STp		
			a+b	c+e	d+e	a+b	f+h	g+h
21	*Enterococcus faecalis*	JCM 6803	-	-	-	-	-	-
22	*Klebsiella pneumoniae*	JCM 1662	-	-	-	-	-	-
23	*Proteus vulgaris*	JCM 1668	-	-	-	-	-	-
24	*Citrobacter freundii*	ATCC 33128	-	-	-	-	-	-
25	*Streptococcus pyogenes*	ATCC 12344	-	-	-	-	-	-
26	*Streptococcus pneumoniae*	ATCC 33400	-	-	-	-	-	-
27	*Haemophilus influenzae*	ATCC 33391	-	-	-	-	-	-
28	*Proteus mirabilis*	ATCC 29906	-	-	-	-	-	-
29	*Neisseria gonorrhoeae*	ATCC 19424	-	-	-	-	-	-
30	*Neisseria meningitidis*	ATCC 13077	-	-	-	-	-	-
31	*Listeria monocytogenes*	ATCC 15313	-	-	-	-	-	-
32	*Lactobacillus acidophilus*	JCM 1132	-	-	-	-	-	-
33	*Bifidobacterium adolescentis*	JCM 1276	-	-	-	-	-	-
34	*Fusobacterium nucleatum*	ATCC 25586	-	-	-	-	-	-
35	*Propionibacterium acnes*	ATCC 6919	-	-	-	-	-	-
36	*Veillonella atypica*	ATCC 17744	-	-	-	-	-	-
37	*Pseudomonas aeruginosa*	IFO 12689	-	-	-	-	-	-
38	*Corynebacterium diphtheriae*	JCM 1310	-	-	-	-	-	-
39	*Peptostreptococcus anaerobius*	ATCC 27337	-	-	-	-	-	-
40	Human placental DNA		-	-	-	-	-	-

Table 57

No.	Name		STh			STp		
			a+b	c+e	d+e	a+b	f+k	g+k
41	*Vibrio cholerae O1*	PB1	·	·	·	·	·	·
42	*Vibrio cholerae O1*	SGN 7277	·	·	·	·	·	·
43	*Vibrio cholerae O1*	11094-78	·	·	·	·	·	·
44	*Vibrio cholerae O1*	E 9120	·	·	·	·	·	·
45	*Vibrio cholerae O1*	E 506	·	·	·	·	·	·
46	*Vibrio cholerae O1*	PB 17	·	·	·	·	·	·
47	*Vibrio cholerae O1*	81H-110	·	·	·	·	·	·
48	*Vibrio cholerae O1*	161H-151	·	·	·	·	·	·
49	*Vibrio cholerae O1*	56H-118	·	·	·	·	·	·

Table 58

No	RPLA	PCR primer combination							PCR primer combination					PCR primer combination				PCR primer combination		
		a+f	b+d	b+e	b+f	c+d	c+e	c+f	g+k	h+j	h+k	h+l	i+k	m+g	n+q	o+q	o+r	g+z	l+z	u+x
1	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
2	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
12	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
14	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
15	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
16	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
17	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
18	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
19	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
23	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
24	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
25	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
26	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
27	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
28	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+

Table 59

No	APLA	PCR primer combination							PCR primer combination					PCR primer combination				PCR primer combination		
		a+f	b+d	b+e	b+f	c+d	c+e	c+f	g+k	h+j	h+k	h+l	i+k	m+g	n+q	o+q	o+r	s+z	t+z	u+x
31	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
32	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
33	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
34	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
35	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
36	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
37	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
41	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
46	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
53	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
54	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
55	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 60

		PCR primer combination							PCR primer combination					PCR primer combination				PCR primer combination		
No.	RPLA	a+f	b+d	b+e	b+f	c+d	c+e	c+f	g+k	h+j	h+k	h+l	i+k	m+g	n+q	o+q	o+r	s+z	t+z	u+x
61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
67	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
68	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
69	A D	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+
70	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
71	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
72	C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
73	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
74	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
75	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
76	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
77	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
78	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
79	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
80	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
82	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
83	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
84	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
85	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
86	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
87	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
88	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
89	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
90	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-

Table 61

No	RPLA	PCR primer combination							PCR primer combination					PCR primer combination				PCR primer combination		
		a+f	b+d	b+e	b+f	c+d	c+e	c+f	g+k	h+j	h+k	h+l	i+k	m+g	n+q	o+q	o+r	g+z	t+z	u+x
91	AB	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
92	AB	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
106	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
107	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
108	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
109	AD	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+
110	AD	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+
111	AD	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+
112	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
113	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
114	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
115	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
116	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
117	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
118	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
119	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
120	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Table 62

		PCR primer combination							PCR primer combination					PCR primer combination				PCR primer combination		
No.	RPLA	a+f	b+d	b+e	b+f	c+d	c+e	c+f	g+k	h+j	h+k	h+l	i+k	m+g	n+q	o+q	o+r	s+z	t+z	u+x
121	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
122	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
124	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
125	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
126	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
127	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
128	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
129	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
131	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
133	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+
134	B	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-
135	C, D	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
136	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
137	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
138	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
139	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
141	A	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
142	B	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-
143	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
144	B	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-
145	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
146	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
148	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
149	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
150	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-

Table 63

No	RPLA	PCR primer combination							PCR primer combination					PCR primer combination				PCR primer combination		
		a+ℓ	b+d	b+e	b+ℓ	c+d	c+e	c+f	g+k	h+j	h+k	h+ℓ	i+k	m+g	n+q	o+q	o+r	s+z	t+z	u+x
151	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
152	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
153	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
154	A B	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
156	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
157	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 64

No.	Name		PCR primer combination							PCR primer combination					PCR primer combination				PCR primer combination		
			a·f	b·d	b·e	b·f	c·d	c·e	c·f	g·k	h·j	h·k	h·l	i·k	m·q	n·q	o·q	p·r	s·z	t·z	u·x
1	Bacillus cereus	ATCC 14579	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Bacillus subtilis	JCM 1465	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Staphylococcus epidermidis	JCM 2414	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Salmonella typhimurium	IFO 12529	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Salmonella enteritidis	IFO 3313	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Clostridium perfringens	ATCC 12917	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Vibrio cholerae	ATCC 25872	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Vibrio cholerae type ogawa	ATCC 9458	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Vibrio cholerae type inaba	ATCC 9459	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Vibrio fluvialis	JCM 3752	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Campylobacter jejuni	JCM 2013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Campylobacter coli	JCM 2529	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	Escherichia coli	JCM 1649	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Yersinia enterocolitica	ATCC 9610	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Shigella dysenteriae	ATCC 9361	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Shigella flexneri	ATCC 29903	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Shigella sonnei	ATCC 29930	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	Bacteroides fragilis	ATCC 23745	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	Bacteroides vulgatus	JCM 5826	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	Enterococcus faecalis	JCM 5803	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	Klebsiella pneumoniae	JCM 1662	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Proteus vulgaris	JCM 1668	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	Citrobacter freundii	ATCC 33128	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	Streptococcus pyogenes	ATCC 12344	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	Streptococcus pneumoniae	ATCC 33400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	Haemophilus influenzae	ATCC 33391	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	Proteus mirabilis	ATCC 29905	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	Neisseria gonorrhoeae	ATCC 19424	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	Neisseria meningitidis	ATCC 13077	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	Listeria monocytogenes	ATCC 15313	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	Lactobacillus acidophilus	JCM 1132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	Bifidobacterium adolescentis	JCM 1275	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	Fusobacterium nucleatum	ATCC 25586	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	Propionibacterium acnes	ATCC 6919	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	Veillonella atypica	ATCC 17744	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	Pseudomonas aeruginosa	IFO 12689	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	Corynebacterium diphtheriae	JCM 1310	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	Peptostreptococcus anaerobius	ATCC 27337	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	Human placental DNA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 65

		Primer combination			
S. aureus strain No.	RPLA	a+d	a+f	b+c	b+e
69	A, D	-	-	-	-
83	A	-	-	-	-
FRI-722	A, D	-	-	-	-
553	A	-	-	-	-
1	A, B	-	-	-	-
FDA-243(ATCC14458)	B	-	-	-	-
213	B	-	-	-	-
505	B	-	-	-	-
35	C	-	-	-	-
72	C	-	-	-	-
361	C, D	-	-	-	-
595	C	-	-	-	-
27	D	-	-	-	-
112	A, D	-	-	-	-
1151-7NG	D	-	-	-	-
542	D	-	-	-	-
FRI-326(ATCC27664)	- (E)	+	+	+	+

Table 66

Primer combination

No.	Name		d+d	d+f	b+C	b+e
1	*Bacillus cereus*	ATCC 14579	-	-	-	-
2	*Bacillus subtilis*	JCM 1465	-	-	-	-
3	*Staphylococcus aureus*	JCM 2413	-	-	-	-
4	*Staphylococcus epidermidis*	JCM 2414	-	-	-	-
5	*Salmonella typhimurium*	IFO 12529	-	-	-	-
6	*Salmonella enteritidis*	IFO 3163	-	-	-	-
7	*Clostridium perfringens*	ATCC 12917	-	-	-	-
8	*Vibrio cholerae*	ATCC 25872	-	-	-	-
9	*Vibrio cholerae type ogawa*	ATCC 9458	-	-	-	-
10	*Vibrio cholerae type Inaba*	ATCC 9459	-	-	-	-
11	*Vibrio fluvialis*	JCM 3752	-	-	-	-
12	*Campylobacter jejuni*	JCM 2013	-	-	-	-
13	*Campylobacter coli*	JCM 2529	-	-	-	-
14	*Escherichia coli*	JCM 1649	-	-	-	-
15	*Yersinia enterocolitica*	ATCC 9610	-	-	-	-
16	*Shigella dysenteriae*	ATCC 9361	-	-	-	-
17	*Shigella flexneri*	ATCC 29903	-	-	-	-
18	*Shigella sonnei*	ATCC 29930	-	-	-	-
19	*Bacteroides fragilis*	ATCC 23745	-	-	-	-
20	*Bacteroides vulgatus*	JCM 5826	-	-	-	-
21	*Enterococcus faecalis*	JCM 5803	-	-	-	-
22	*Klebsiella pneumoniae*	JCM 1662	-	-	-	-
23	*Proteus vulgaris*	JCM 1668	-	-	-	-
24	*Citrobacter freundii*	ATCC 33128	-	-	-	-
25	*Streptococcus pyogenes*	ATCC 12344	-	-	-	-
26	*Streptococcus pneumoniae*	ATCC 33400	-	-	-	-
27	*Haemophilus influenzae*	ATCC 33391	-	-	-	-
28	*Proteus mirabilis*	ATCC 29906	-	-	-	-
29	*Neisseria gonorrhoeae*	ATCC 19424	-	-	-	-
30	*Neisseria meningitidis*	ATCC 13077	-	-	-	-
31	*Listeria monocytogenes*	ATCC 15313	-	-	-	-
32	*Lactobacillus acidophilus*	JCM 1132	-	-	-	-
33	*Bifidobacterium adolescentis*	JCM 1275	-	-	-	-
34	*Fusobacterium nucleatum*	ATCC 25586	-	-	-	-
35	*Propionibacterium acnes*	ATCC 6919	-	-	-	-
36	*Veillonella atypica*	ATCC 17744	-	-	-	-
37	*Pseudomonas aeruginosa*	IFO 12689	-	-	-	-
38	*Corynebacterium diphtheriae*	JCM 1310	-	-	-	-
39	*Peptostreptococcus anaerobius*	ATCC 27337	-	-	-	-
40	Human placental DNA		-	-	-	-

Claims

1. An oligonucleotide which is complementary to nucleic acid which comprises a target sequence encoding the thermostable direct hemolysin-related hemolysin gene type 1 or type 2 (trh1 trh2 gene) of

Vibrio parahaemolyticus, said oligonucleotide comprising a sequence selected from among the sequences:
(5') d-GGCTCAAAATGGTTAAGCG (3') (a: SEQ ID NO;1)
and
(5') d-CATTTCCGCTCTCATATGC (3') (b: SEQ ID NO;2)
or from among the corresponding complementary sequences.

2. An oligonucleotide which is complementary to nucleic acid which comprises a target sequence encoding the thermostable direct hemolysin gene (tdh gene) of Vibrio parahaemolyticus, said oligonucleotide comprising a sequence selected from among the sequences:
(5') d-CCATCTGTCCCTTTTCCTGC (3') (c: SEQ ID NO;3)
(5') d-CCAAATACATTTTACTTGG (3') (d: SEQ ID NO;4)
(5') d-GGTACTAAATGGCTGACATC (3') (e: SEQ ID NO;5)
and
(5') d-CCACTACCACTCTCATATGC (3') (f: SEQ ID NO;6)
or from among the corresponding complementary sequences.

3. An oligonucleotide which is complementary to nucleic acid which comprises a target sequence encoding the thermostable direct hemolysin gene type 1 (trh1 gene) of Vibrio parahaemolyticus, said oligonucleotide comprising a sequence selected from among the sequences:
(5') d-GGCTCAAAATGGTTAAGCGC (3') (g: SEQ ID NO;7)
and
(5') d-TGGCGTTTCATCCAAATACG (3') (h: SEQ ID NO;8)
or from among the corresponding complementary sequences.

4. A method of detecting Vibrio parahaemolyticus in a test sample by selectively amplifying a target nucleotide sequence using, as primers for chain extension reaction, at least one pair of oligonucleotides each defined in claim 1, 2 or 3, said method comprising
(a) hybridizing the primers to the target sequence occurring in the single-stranded form in the sample and causing chain extension by the polymerization reaction involving four nucleotide triphosphates (dNTPs),
(b) separating the thus-obtained double-stranded target nucleotide sequence into single strands, each complementary strand being capable of functioning as a template for chain extension reaction involving a complementary primer,
(c) repeating the chain extension reaction involving the primers, separating primer chain extension products from the templates and hybridizing with primer molecules thereby to amplify the target sequence, and detecting the thus-amplified nucleotide fragment, and
(d) judging, based on the detection result, whether the sequence to be recognized is present in said sample.

5. An oligonucleotide which is complementary to nucleic acid which comprises a target sequence encoding the heat-liable toxin (LT) produced by toxigenic Escherichia coli, said oligonucleotide comprising all or part of a sequence selected from among the sequences
(5') d-CCCAGATGAAATAAAACGT-(3') (a: SEQ ID NO;9)
(5') d-CCTGAGATATATTGTGCTC-(3') (b: SEQ ID NO;10)
(5') d-ACAAACCGGCTTTGTCAGATAT-(3') (c: SEQ ID NO;11)
(5') d-GTTATATATGTCAACCTCTGAC-(3') (d: SEQ ID NO;12)
and
(5') d-ACCGGTATTACAGAAATCTGA-(3') (e: SEQ ID NO;13)
or from among the corresponding complex sequences.

6. A method of detecting toxigenic Escherichia coli in a test sample by selectively amplifying a target nucleotide sequence using, as primers for chain extension reaction, at least one pair of oligonucleotides each defined in claim 5, said method comprising
(a) hybridizing the primers to the target sequence occurring in the single-stranded form in the sample and causing chain extension by the polymerization reaction involving four nucleotide triphosphates (dNTPs),

(b) separating the thus-obtained double-stranded target nucleotide sequence into single strands, each complementary strand being capable of functioning as a template for chain extension reaction involving a complementary primer,
(c) repeating the chain extension reaction involving the primers, separating primer chain extension products from the templates and hybridizing with primer molecules thereby to amplify a target sequence, and detecting the thus-amplified nucleotide fragment, and
(d) judging, based on the detection result, whether the sequence to be recognized is present in said sample.

7. An oligonucleotide which is complementary to nucleic acid and which comprises a target sequence encoding the human thermostable enterotoxin (STh) gene or porcine thermostable enterotoxin (STp) gene of enterotoxigenic Escherichia coli said oligonucleotide comprising all or part of a sequence selected from among the sequences
(5') d-TGTAATTTTCTCTTTTGAAGACTC-(3') (a: SEQ ID NO;14)
and
(5') d-ATTACAACACAGTTCACAGCAG-(3') (b: SEQ ID NO;15)
or from among the corresponding complementary sequences.

8. An oligonucleotide which is complementary to nucleic acid which comprises, a target sequence encoding the human thermostable enterotoxin (STh) gene of enterotoxigenic Escherichia coli, said oligonucleotide comprising a sequence selected from among the sequences
(5') d-CCTCAGGATGCTAAACCAG-(3') (c: SEQ ID NO;16)
(5') d-AGGATGCTAAACCAGTAGAG-(3') (d: SEQ ID NO;17)
and
(5') d-AATTCACAGCAGTAATTGCTAC-(3') (e: SEQ ID NO;18)
or from among the corresponding complementary sequences.

9. An oligonucleotide which is complementary to nucleic acid which comprises, a nucleotide sequence encoding the porcine thermostable enterotoxin (STp) gene of enterotoxigenic Escherichia coli, said oligonucleotide comprising a sequence selected from among the sequences
(5') d-TCTTTCCCCTCTTTTAGTCAG-(3') (f: SEQ ID NO;19)
(5') d-GTCAACTGAATCACTTGACTC-(3') (g: SEQ ID NO;20)
and
(5') d-TCACAGCAGTAAAATGTGTTG-(3') (e: SEQ ID NO;21)
or from among the corresponding complementary sequences.

10. A method of detecting STh- and/or STp-producing Escherichia coli in a test sample by selectively amplifying a target nucleotide sequence using, as primers for chain extension reaction, at least one pair of oligonucleotides each defined in claim 7, 8 or 9, said method comprising
(a) hybridizing the primers to the target sequence occurring in the single-stranded form in the sample and causing chain extension by the polymerization reaction involving four nucleotide triphosphates (dNTPs),
(b) separating the thus-obtained double-stranded target nucleotide sequence into single strands, each complementary strand being capable of functioning as a template for chain extension reaction involving a complementary primer,
(c) repeating the chain extension reaction involving the primers, separating primer chain extension products from the templates and hybridizing with primer molecules thereby to amplify the target nucleotide sequence, and detecting the thus-amplified nucleotide fragment, and
(d) judging, based on the detection result, whether the sequence to be recognized is present in said sample.

11. An oligonucleotide which is complementary to nucleic acid which comprises a target nucleotide sequence encoding the gene (entA gene) for enterotoxin A produced by Staphylococcus aureus, said oligonucleotide comprising a sequence selected from among the sequences
(5') d-GTCTGAATTGCAGGGAACAG-(3') (a: SEQ ID NO;22) ,
(5') d-CTTTTTTACAGATCATTCGTG-(3') (b: SEQ ID NO;23) ,
(5') d-TAGATTTTGATTCAAAGGATATTG-(3') (c: SEQ ID NO;24) ,
(5') d-CTTATTCGTTTTAACCGTTTCC-(3') (d: SEQ ID NO;25) ,

(5') d-AACACGATTAATCCCCTCTG-(3') (e: SEQ ID NO;26) ,
(5') d-TCGTAATTAACCGAAGGTTCTG-(3') (f: SEQ ID NO; 27) ,
or from among the corresponding complementary sequences.

12. An oligonucleotide which is complementary to nucleic acid which comprises a target nucleotide sequence encoding the gene (entB gene) for enterotoxin B produced by Staphylococcus aureus, said oligonucleotide comprising a sequence selected from among the sequences
(5') d-AAATCTATAGATCAATTTCTATAC-(3') (g: SEQ ID NO;28) ,
(5') d-AATTATGATAATGTTCGAGTCG-(3') (h: SEQ ID NO;29) ,
(5') d-TTCGCATCAAACTGACAAACG-(3') (i: SEQ ID NO;30) ,
(5') d-CATCTTCAAATACCCGAACAG-(3') (j: SEQ ID NO;31) ,
(5') d-CCAAATAGTGACGAGTTAGG-(3') (k: SEQ ID NO;32) ,
(5') d-TCATACCAAAAGCTATTCTCAT-(3') (l: SEQ ID NO;33) ,
or from among corresponding complementary sequences.

13. An oligonucleotide which is complementary to nucleic acid which comprises a target nucleotide sequence encoding the gene (entC gene) for enterotoxin C produced by Staphylococcus aureus, said oligonucleotide comprising a sequence selected from among the sequences
(5') d-TCTGTAGATAAATTTTTGGCA-(3') (m: SEQ ID NO;34) ,
(5') d-AAAATTATGACAAAGTGAAAACAG-(3') (n: SEQ ID NO;35) ,
(5') d-ATGGATCAAATTACTATGTAAAC-(3') (o: SEQ ID NO;36) ,
(5') d-GTAGGTAAAGTTACAGGTGG-(3') (p: SEQ ID NO;37) ,
(5') d-TATAAGTACATTTTGTAAGTTCC-(3') (q: SEQ ID NO;38) ,
(5') d-CATACCAAAAAGTATTGCCGTT-(3') (r: SEQ ID NO;39) ,
or from among the corresponding complementary sequences.

14. An oligonucleotide which is complementary to nucleic acid which comprises a target sequence encoding the gene (entD gene) for enterotoxin D produced by Staphylococcus aureus, said oligonucleotide comprising a sequence selected from among the sequences
(5') d-AAAATCTGAATTAAGTAGTACCG-(3') (s: SEQ ID NO;40) ,
(5') d-ATAGGAGAAAATAAAAGTACAGG-(3') (t: SEQ ID NO;41) ,
(5') d-CTTCAATTCAAAAGAAATGGC-(3') (u: SEQ ID NO;42) ,
(5') d-TTGTACATATGGAGGTGTCAC-(3') (v: SEQ ID NO;43) ,
(5') d-TTTTAGATTTGAAATGTTGAGCC-(3') (w: SEQ ID NO;44) ,
(5') d-TGACACCTCCATATGTACAAG-(3') (x: SEQ ID NO;45) ,
(5') d-ATTATACAATTTTAAATCCTTTTGC-(3') (y: SEQ ID NO;46) ,
(5') d-CTGTATTTTTCCTCCGAGAGT-(3') (z: SEQ ID NO;47) ,
or from among the corresponding complementary sequences.

15. An oligonucleotide which is complementary to nucleic acid which comprises a target nucleotide sequence encoding the gene (entE gene) for enterotoxin E produced by Staphylococcus aureus, said oligonucleotide comprising a sequence selected from among the sequences
(5')d-AAAAGTCTGAATTACAAAGAAATG-(3') (a:SEQ ID NO;48),
(5')d-GGTTTTTTCACAGGTCATCCA-(3') (b:SEQ ID NO;49),
(5')d-GAACAGTTACTTCTTTTTTGCTT-(3') (c:SEQ ID NO;50),
(5')d-CTGTCTGAGTTATATAAACCAA-(3') (d:SEQ ID NO;51),
(5')d-GCACCTTACCGCCAAAGCTG-(3') (e:SEQ ID NO;52),
(5')d-AAACAAATCATAACTTACCGTG-(3') (f:SEQ ID NO;53),
or from among the corresponding complementary sequences.

16. A method of detecting Staphylococcus aureus in a test sample by selectively amplifying a target nucleotide sequence using, as primers for chain extension reaction, at least one pair of oligonucleotides each defined in claim 11, 12, 13, 14 or 15, said method comprising.
(a) hybridizing the primers to the target nucleotide sequence occurring in the single-stranded form in the sample and causing chain extension by the polymerization reaction involving four nucleotide triphosphates (dNTPs),
(b) separating the thus-obtained double-stranded target nucleotide sequence into single strands, each complementary strand being capable of functioning as a template for chain extension reaction

involving a complementary primer,
(c) repeating the chain extension reaction involving the primers, separating primer chain extension products from the templates and hybridizing with new primer molecules thereby to amplify the target nucleotide sequence, and detecting the thus-amplified nucleotide fragment, and
(d) judging, based on the detection result, whether the sequence to be recognized is present in said sample.

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(54) Oligonucleotides for detecting bacteria.

(57) Oligonucleotides (SEQ ID NOs 1-8) selectively hybridizable with a specific gene of Vibro parahaemolyticus, oligonucleotides (SEQ ID NOs 9-13) selectively hybridizable with the LT gene of toxigenic Escherchia coil, oligonucleotides (SEQ ID NOs 14-21) selectively hybrizable with the STh or STp gene of toxigenic Escherchia coil, oligonucleotides (SEQ ID NOs 22-47) selectively hybridizable with the entA, B, C, or D gene of Staphylococcus aureus, or oligonucleotides (SEQ ID NOs 48-53) selectively hybridizable with the entE gene of Staplyloccus aureus are prepared and used as primers for gene amplification to thereby selectively detect only respective microorganisms causing food poisoning.

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European Patent Office

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Application Number
EP 92 30 7606

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
X	DATABASE WPI Week 9210, Derwent Publications Ltd., London, GB; AN 92-076290 & JP-A-4 020 299 (TOYOBO KK) 23 January 1992 * abstract * ---	1,3,4	C12Q1/68
X	JOURNAL OF CLINICAL MICROBIOLOGY, vol.23, no.6, June 1986, WASHINGTON US pages 1091 - 1095 M.NISHIBUCHI ET AL. * page 1092; table 1 * ---	2,4	
D,A	INFECTION AND IMMUNITY, vol.57, no.9, September 1989, BETHESDA US pages 2691 - 2697 M.NISHIBUCHI ET AL. * the whole document * ---	1	
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X	EP-A-0 409 159 (SHIMADZU CORPORATION) * page 11, line 15 - page 14, line 11 * * page 24, line 56 - page 31, line 26 * * page 34, line 25 - page 36, line 55 * * claims 5,6,8,24 *	5-10,12,16	
A	---	2,4	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	25 February 1994	De Kok, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
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E : earlier patent document, but published on, or after the filing date
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EUROPEAN SEARCH REPORT

Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
X	JOURNAL OF CLINICAL MICROBIOLOGY, vol.29, no.11, November 1991, WASHINGTON US pages 2375 - 2379 E.HORNES ET AL. * page 2375 - page 2377 * ---	8,10	
X	EP-A-0 265 244 (AMOCO CORPORATION) * page 11, line 35 - line 61 * ---	5,6	
A	DATABASE WPI Derwent Publications Ltd., London, GB; AN 91-183000 & JP-A-3 112 499 (SHIMADZU CORP.) 14 May 1991 * abstract * ---	7-10	
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A	AMERICAN JOURNAL OF VETERINARY RESEARCH, vol.46, no.4, April 1985, CHICAGO US pages 909 - 912 T.SEKIZAKI ET AL. * the whole document * ---	7-10	
A	APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol.57, no.6, June 1991, WASHINGTON US pages 1793 - 1798 I.G.WILSON ET AL. * the whole document * ---	11-16	
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The present search report has been drawn up for all claims

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 92 30 7606

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
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E	WO-A-92 17609 (M.J. HOLMES ET AL.) * page 15, line 9 - line 37 *	8,10	
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E	DATABASE WPI Week 9248, Derwent Publications Ltd., London, GB; AN 92-394404 & JP-A-4 293 486 (SHIMADZU CORP) 19 October 1992 * abstract *	1,4	TECHNICAL FIELDS SEARCHED (Int.Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	25 February 1994	De Kok, A

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D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- [ ] All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- [ ] Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.

  namely claims:
- [ ] No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

[X] LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,
namely:

1. Claims 1-4 : Method and oligonucleotides for the detection of Vibrio parahaemolyticus.
2. Claims 5,6 : Method and oligonucleotides for the detection of toxigenic E. coli.
3. Claims 7-10 : Method and oligonucleotides for the detection of STh- or STp-producing E. coli.
4. Claims 11-16 : Method and oligonucleotides for the detection of S. Aureus.

- [x] All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- [ ] Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

  namely claims:
- [ ] None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

  namely claims: